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Altered metabolic profiles in male offspring conceived from intracytoplasmic sperm injection

Bingqian Zhang[1,3,4,5,6,7,8,9,10,13], Miaomiao Ban[1,4,5,6,7,8,9,10], Xiaojing Chen[1,4,5,6,7,8,9,10], Jingmei Hu[1,4,5,6,7,8,9,10], Linlin Cui[2,4,5,6,7,8,9,10*] and Zi-Jiang Chen[1,2,4,5,6,7,8,9,10,11,12]

Abstract

Background While most research has focused on the association between intracytoplasmic sperm injection (ICSI) and neurodevelopmental disorders in children, relatively little attention has been given to its metabolic effects. Previous studies have reported that low serum lipid levels are associated with mental health problems. Our objective was to analyze the impact of ICSI on metabolic alterations compared to their in vitro fertilization (IVF) counterparts in male offspring, as well as its interaction with paternal overweight/obesity.

Methods We recruited families between January 2006 and December 2017 at the Center for Reproductive Medicine, Shandong University, China. Prospective data of offspring were obtained for body mass index (BMI), blood pressure, glucose, and lipid profile in their 0–11 years old. Linear mixed models were utilized to compute the mean difference and 95% confidence intervals (CI).

Results A total of 14,196 offspring visits were identified. In offspring aged 4–11 years, ICSI-conceived offspring exhibited significantly lower fasting glucose *z*-scores, total cholesterol *z*-scores, and low-density lipoprotein cholesterol (LDL-C) *z*-scores compared with their IVF counterparts (fasting glucose *z*-score: adjusted mean difference: − 0.13, 95% CI: − 0.23 to − 0.03; total cholesterol *z*-score: adjusted mean difference: − 0.13, 95% CI: − 0.23 to − 0.02; LDL-C *z*-score: adjusted mean difference: − 0.12, 95% CI: − 0.22 to − 0.01). Paternal overweight/obesity significantly influenced the relationship between ICSI and metabolic changes in offspring. In offspring born from fathers with overweight/obesity, ICSI-conceived offspring displayed significantly lower fasting glucose and total cholesterol *z*-scores than their IVF controls (fasting glucose *z*-score: adjusted mean difference: − 0.20, 95% CI: − 0.32 to − 0.08; total cholesterol *z*-score: adjusted mean difference: − 0.15, 95% CI: − 0.27 to − 0.02). In offspring born to fathers with normal weight, ICSI-conceived offspring showed significantly lower systolic blood pressure *z*-scores compared to those conceived via the IVF procedures (adjusted mean difference: − 0.21, 95% CI: − 0.37 to − 0.05).

Conclusions The findings of this study suggested that ICSI was associated with altered glucose and lipid profiles compared to their IVF controls, characterized by lower fasting glucose *z*-scores, total cholesterol *z*-scores, and LDL-C *z*-scores. Encouraging fathers to reduce their body weight could potentially improve the metabolic health of their ICSI-conceived children.

Keywords Paternal obesity, Intracytoplasmic sperm injection, In vitro fertilization, Metabolic profiles

*Correspondence:
Linlin Cui
liy@sdu.edu.cn
Full list of author information is available at the end of the article

Background

The increasing using of assisted reproductive technology (ART) worldwide carries a significant rise in concerns about the safety of their offspring [1–3]. Intracytoplasmic sperm injection (ICSI) is a well-established laboratory technique for fertilization in ART, which involves the injection of a single spermatozoon (or sperm head or nucleus) into an oocyte cytoplasm. It is commonly performed when the males have a severe abnormality of semen parameters, fertilization deficiency, and a number of non-male factor indications [4–6]. Recent data from the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) indicate that ICSI is utilized in two-thirds of all fresh ART cycles globally, with approximately 55% of ART procedures in Asia being ICSI cycles [7]. However, concerns persist regarding the safety of offspring conceived through ICSI due to the invasive nature of the procedures involved.

The manipulation of gametes in ICSI occurs within a critical developmental window that influences genomic methylation, which in turn associated with the future development of children [8, 9]. Several studies have indicated an elevated risk of neurodevelopmental disorders in offspring born through the ICSI procedure [10, 11]. However, it is widely recognized that specific metabolic traits or phenotypes can shape the distinct cellular and biochemical properties of the nervous system, from its embryonic formation to its functioning in adulthood [12]. For instance, low blood glucose and cholesterol levels have been associated with an increased risk of autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) in children, potentially involving imbalanced cortisol levels, energy deficiency, and mitochondrial dysfunction [13]. The mechanism might involve unbalanced cortisol levels, energy deprivation, and mitochondrial dysfunction. Yet, limited studies examining the metabolic profiles of children conceived through ICSI, particularly in comparison with conventional IVF controls, both of which involve embryo culture in vitro. Recently, Catford et al. reported a comparable metabolic profile between ICSI- and IVF-conceived men with a relatively lower recruitment rate [14]. Therefore, it is imperative to assess the associations between ICSI and metabolic alterations in offspring.

A key issue is whether metabolic changes in children conceived through ICSI are due to the ICSI procedure itself or to parents' poor metabolic health, such as obesity [2, 15]. It is reported that paternal or maternal obesity before pregnancy is associated with an increased risk for obesity, higher blood pressure, and insulin resistance in their offspring [16–18]. Hence, it is crucial to investigate the differences in offspring metabolic profiles between infertile parents who are overweight or obese and those who undergo ICSI treatment. However, existing studies have not explored the associations between paternal overweight/obesity, ICSI, and offspring metabolic alterations.

Here, we conducted a hospital-based cohort study aimed at elucidating the impact of ICSI on the metabolic profiles of offspring compared to IVF, considering its interaction with paternal body mass index (BMI) status. The findings of this study will offer a definitive statement regarding the metabolic risks in offspring conceived through ICSI.

Methods

Study design and setting

This study was a prospective cohort study conducted at the Center for Reproductive Medicine, Cheeloo College of Medicine, Shandong University, China. We recruited families who underwent assisted reproductive technology (ART) treatment between January 2006 and December 2017. Families who conceived using in vitro fertilization/intracytoplasmic sperm injection (IVF/ICSI) procedures were included. Exclusion criteria included parents who used oocyte or sperm donation ($N=1140$), families with incomplete anthropometric information ($N=212$), the male partners were underweight ($N=209$), and families who did not participate in the birth cohort were excluded ($N=2168$). Prospective data of offspring were obtained for BMI, blood pressure, glucose, and lipid profile during 2014–2021. Information was collected at birth and follow-ups at ages 0, 6 months, 1–2 years, 3–4 years, 5–6 years, 7–9 years, and above 10 years. A total of 15,415 offspring with 27,698 visits were identified. We excluded offspring aged 11 years or older and female offspring. Finally, a total of 7816 male offspring with 14,196 visits were included in our study (Additional file 1: Fig. S1). We followed the guidelines on Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement in reporting our study.

Exposure

We divided offspring into two groups to investigate the risks of ICSI in metabolic alterations in offspring: (1) ICSI conception and (2) IVF conception. Subsequently, we evaluated metabolic outcomes in offspring based on types of ART therapy and paternal BMI status, distinguishing between (1) ICSI conception and paternal overweight/obesity, (2) IVF conception and paternal overweight/obesity, (3) ICSI conception and paternal normal weight, and (4) IVF conception and paternal normal weight. Furthermore, we determined whether the offspring's metabolic outcomes were influenced by the following factors: (1) singleton births only and (2) percutaneous epididymal sperm aspiration (PESA)/

testicular sperm extraction (TESA)-ICSI methods versus traditional ICSI methods. The categories of paternal and maternal pre-pregnancy BMI were defined according to the Working Group on Obesity in China as follows: obesity/overweight (BMI ≥ 24.0 kg/m^2) and normal weight (18.5 kg/m^2 ≤ BMI < 24.0 kg/m^2) [19, 20].

Measurements

Offspring physical examinations of the children were conducted by pediatric physicians and nurses at each visit. Height (± 0.1 cm), weight (± 0.1 kg), and chest/waist circumference were measured twice, and the average value was recorded. Blood pressure was measured on the right arm of the seated child by nurses. Three blood pressure readings were taken, and the average of the last two readings was utilized. Blood tests were conducted after an overnight fast. Following standard operating procedures, all blood samples were immediately centrifuged after collection and temporarily stored at 4 °C. Within 24 h, the samples were distributed to our Biobank, where they were stored at − 80 °C until analysis. The children's fasting glucose, fasting insulin, triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were measured as previously reported [21, 22].

Outcomes

The outcomes were the age- and sex-specific *z*-scores of cardiometabolic variables in offspring conceived with IVF/ICSI. We analyzed the between-group mean difference of offspring BMI *z*-score, systolic and diastolic blood pressure *z*-score, glucose *z*-score, insulin *z*-score, homeostatic model assessment for insulin resistance (HOMA-IR) *z*-score, triglyceride *z*-score, total cholesterol *z*-score, LDL-C *z*-score, and HDL-C *z*-score. The offspring BMI and offspring HOMA-IR were defined and calculated as previously described [21, 22]. The age- and sex-specific (every 6 months) *z*-score was calculated as [(observed value minus age- and sex-specific mean)/(age- and sex-specific SD)], using data from the total number of ART-conceived offspring conceived at our hospital [23].

Statistical analysis

All statistical tests were performed using R software version 3.6.3. For baseline characteristics, continuous variables with normally distributed are expressed as mean ± SD, and differences are tested by one-way ANOVA and *t*-test; continuous variables with non-normally distributed are expressed as median [interquartile range (IQR)], and differences are tested by Wilcoxon rank-sum test. Categorical variables are summarized as frequency (percentage), and differences are performed by χ^2 analysis.

As this study included a repeated-measures design, we utilized a linear mixed model to account for both random and fixed effects for the time-dependent and time-independent variables, respectively. The random effect included the unique offspring ID number. The fixed effects included ICSI treatment, paternal BMI status, paternal age, maternal age, maternal BMI, parity, plurality, parental smoking, frozen embryo transfer, and offspring age. The interaction *P* value was calculated, and $P < 0.05$ was considered significant difference. To validate the assumptions of our linear mixed model, we conducted tests for the normality of residuals, homogeneity of variances, and independence of observations. Normality of residuals was assessed using histogram plots, homogeneity of variances was evaluated using residual plots against fitted values, and independence of observations was checked using autocorrelation plots of residuals. The power analysis was calculated using the R package "simr" for the linear mixed models, which were based on Monte Carlo simulations. Our analysis could detect an absolute effect size of 0.17 in the fasting glucose *z*-score between the IVF and ICSI treatment groups using a linear mixed model, with 88% power at a significance level of 0.05. The post hoc analysis was calculated between groups and 95% confidence interval (CI) and $P < 0.025$ was considered statistical different (Bonferroni correction, $P = 0.05/n$).

Results

Characteristics of the study population

We identified a total of 14,196 offspring visits conceived through IVF/ICSI. Among these, 9642 (67.92%) were born from fathers with overweight/obesity, while 4554 (32.08%) were born from fathers with normal weight. In comparison to IVF-conceived offspring, those conceived through ICSI exhibited significantly lower paternal age, paternal BMI, maternal age, maternal BMI, and incidence of parental smoking. Conversely, the proportion of first-born children was significantly higher among ICSI-conceived offspring (Table 1). Additionally, the number of oocytes retrieved, good-quality embryos on day 3, and frozen embryo transfer were significantly higher in the ICSI-conceived group, while the total gonadotropin dose was significantly lower (Additional file 2: Table S1). The mean age of the offspring was 2.35 ± 1.94 years old.

ICSI treatment and offspring metabolic changes

Firstly, we accessed the relationship between ICSI treatment and offspring metabolic changes. After adjusting for paternal age, maternal age, maternal BMI, parity, plurality, parental smoking, frozen embryo transfer, and offspring age, offspring BMI *z*-score, waist-to-height ratio *z*-score, systolic blood pressure *z*-score, and diastolic

Table 1 Characteristics of the study population

	ICSI ($n=4748$)	IVF ($n=9448$)	
Parental characteristics			
Paternal BMI status			
Overweight/obesity	3145 (66.2%)	6497 (68.8%)	< 0.01*
Normal weight	1603 (33.8%)	2951 (31.2%)	
Paternal BMI, kg/m^2	25.88 ± 3.92	26.05 ± 3.97	< 0.01*
Paternal age, years	31.19 ± 4.79	31.73 ± 4.71	< 0.01*
Maternal age, years	30.28 ± 4.26	30.91 ± 4.13	< 0.01*
Maternal BMI, kg/m^2	23.57 ± 3.28	23.82 ± 3.36	< 0.01*
Paternal smoking, no./total no. (%)	1448 (30.5%)	3268 (34.6%)	< 0.01*
Paternal or maternal education level (college or higher), *n* (%)	1197 (25.2%)	2298 (24.3%)	0.25
Parity, no./total no. (%)			
1	3893 (82.0%)	7292 (77.2%)	< 0.01*
2	855 (18.0%)	2156 (22.8%)	
Gestational diabetes mellitus, no./total no. (%)	589 (12.4%)	1124 (11.9%)	0.39
Pregnancy induced hypertension, no./total no. (%)	508 (10.7%)	916 (9.7%)	0.06
Offspring's characteristics			
Age, years			
0–1, *n* (%)	2304 (48.5%)	4410 (46.7%)	0.1
2–3, *n* (%)	1718 (36.2%)	3518 (37.2%)	
4–11, *n* (%)	726 (15.3%)	1520 (16.1%)	
Mean ± SD	2.33 ± 1.94	2.36 ± 1.94	0.39
Plurality, *n* (%)			
Singleton	3715 (78.2%)	7254 (76.8%)	0.05
Multiple	1033 (21.8%)	2194 (23.2%)	
Birthweight (g)	3324.0 ± 610.5	3315.0 ± 604.5	0.41

Continuous variables with normally distributed are expressed as mean ± SD, and differences are tested by *t*-test. Categorical variables are summarized as frequency (percentage), and differences are performed by χ^2 analysis

ICSI, Intracytoplasmic sperm injection, *IVF* In vitro fertilization, *BMI* Body mass index

* Significant difference: $P < 0.05$

blood pressure *z*-score were comparable between ICSI- and IVF-conceived offspring at 0–1 year, 2–3 years, and 4–11 years, respectively. Interestingly, in offspring aged 4–11 years, ICSI-conceived offspring exhibited significantly lower fasting glucose *z*-scores, total cholesterol *z*-scores, and LDL-C *z*-scores than their IVF counterparts (fasting glucose *z*-score: adjusted mean difference: − 0.13, 95% CI: − 0.23 to − 0.03; total cholesterol *z*-score: adjusted mean difference: − 0.13, 95% CI: − 0.23 to − 0.02; LDL-C *z*-score: adjusted mean difference: − 0.12, 95% CI: − 0.22 to − 0.01). There was no significant difference of triglyceride *z*-score and HDL *z*-score between ICSI- and IVF-conceived offspring (Fig. 1, Additional file 3: Tables S2–S3, Fig. S2). To determine whether the mean lipid levels fall within the normal range for children of this age, we evaluated the age-specific total cholesterol, LDL-C, and HDL-C levels in ICSI- and IVF-conceived children in our study. Additionally, we provided our data in both mmol/L and mg/dL units to facilitate easier comparison with previous studies. Our results showed that the total cholesterol and LDL-C levels were lower in ICSI-conceived children compared to IVF-conceived children, and the gap gradually increased with age (Additional file 3: Table S4). In singleton analysis, we found a significantly lower fasting glucose *z*-score and triglyceride *z*-score in offspring conceived from ICSI treatment compared with IVF treatment (fasting glucose *z*-score: adjusted mean difference: − 0.19, 95% CI: − 0.30 to − 0.07; triglyceride *z*-score: adjusted mean difference: − 0.12, 95% CI: − 0.23 to − 0.01) (Additional file 3: Tables S5–S6).

Paternal overweight/obesity, ICSI treatment, and offspring metabolic changes

We then explored the potential mediating role of paternal BMI status on the metabolic changes observed in ICSI-conceived offspring. Among offspring born from fathers with overweight/obesity, we identified 3145 (32.75%) offspring visits conceived through ICSI treatment. In

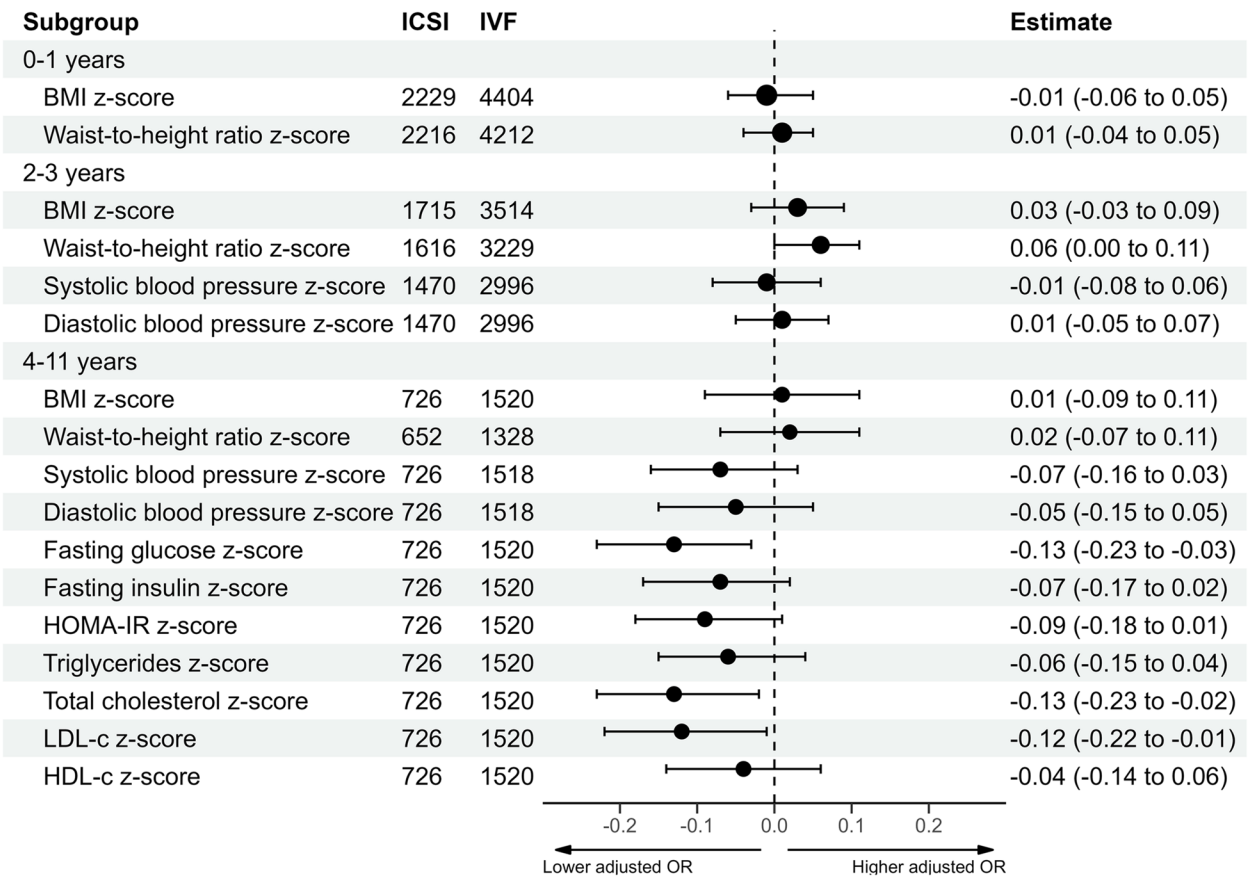

Fig. 1 Associations between ICSI treatment and offspring metabolic alterations

offspring aged 4–11 years with overweight/obese fathers, fasting glucose *z*-scores and total cholesterol *z*-scores were significantly lower in ICSI-conceived offspring compared to IVF-conceived offspring (fasting glucose *z*-score: adjusted mean difference: − 0.20, 95% CI: − 0.32 to − 0.08; total cholesterol *z*-score: adjusted mean difference: − 0.15, 95% CI: − 0.27 to − 0.02) (Fig. 2, Additional file 4: Fig. S3, Tables S7–S8). Similarly, consistent results in fasting glucose *z*-scores were found in singleton offspring (adjusted mean difference: − 0.24, 95% CI: − 0.38 to − 0.10) (Additional file 4: Tables S9–S10). In offspring born from fathers with normal weight, we identified 1603 (35.19%) offspring visits conceived through ICSI treatment. ICSI-conceived offspring exhibited a significantly lower systolic blood pressure *z*-score compared to IVF-conceived offspring aged 4–11 years (adjusted mean difference: − 0.21, 95% CI: − 0.37 to − 0.05) (Fig. 3, Additional file 4: Tables S7–S8). Similar results were observed in singleton offspring (adjusted mean difference: − 0.25, 95% CI: − 0.45 to − 0.05) (Additional file 4: Tables S9–S10).

Given the potential epigenetic effects of traditional ICSI methods versus gametes obtained from PESA/TESA-ICSI treatment, we investigated the associations of paternal overweight/obesity, ICSI methods, and offspring metabolic changes. We identified a total of 837 offspring visits conceived through PESA/TESA-ICSI treatment. In offspring born from paternal overweight/obesity, offspring conceived through PESA/TESA-ICSI appeared to have lower HDL-C *z*-scores than those conceived via traditional ICSI methods (adjusted mean difference: − 0.30, 95% CI: − 0.56 to − 0.03). However, no significant differences in other metabolic changes were found between PESA/TESA-ICSI and traditional ICSI treatment in both the paternal overweight/obesity and paternal normal weight groups (Additional file 5: Tables S11–S12).

Discussion

Our study discovered that ICSI treatment was associated with changes in the metabolic profile of male offspring compared with their IVF controls, manifesting as lower fasting glucose *z*-scores, total cholesterol *z*-scores, and LDL-C *z*-scores. Paternal overweight/obesity played a significant mediating role in the associations between ICSI and offspring metabolic alterations. Among offspring born to fathers with overweight/obesity, offspring

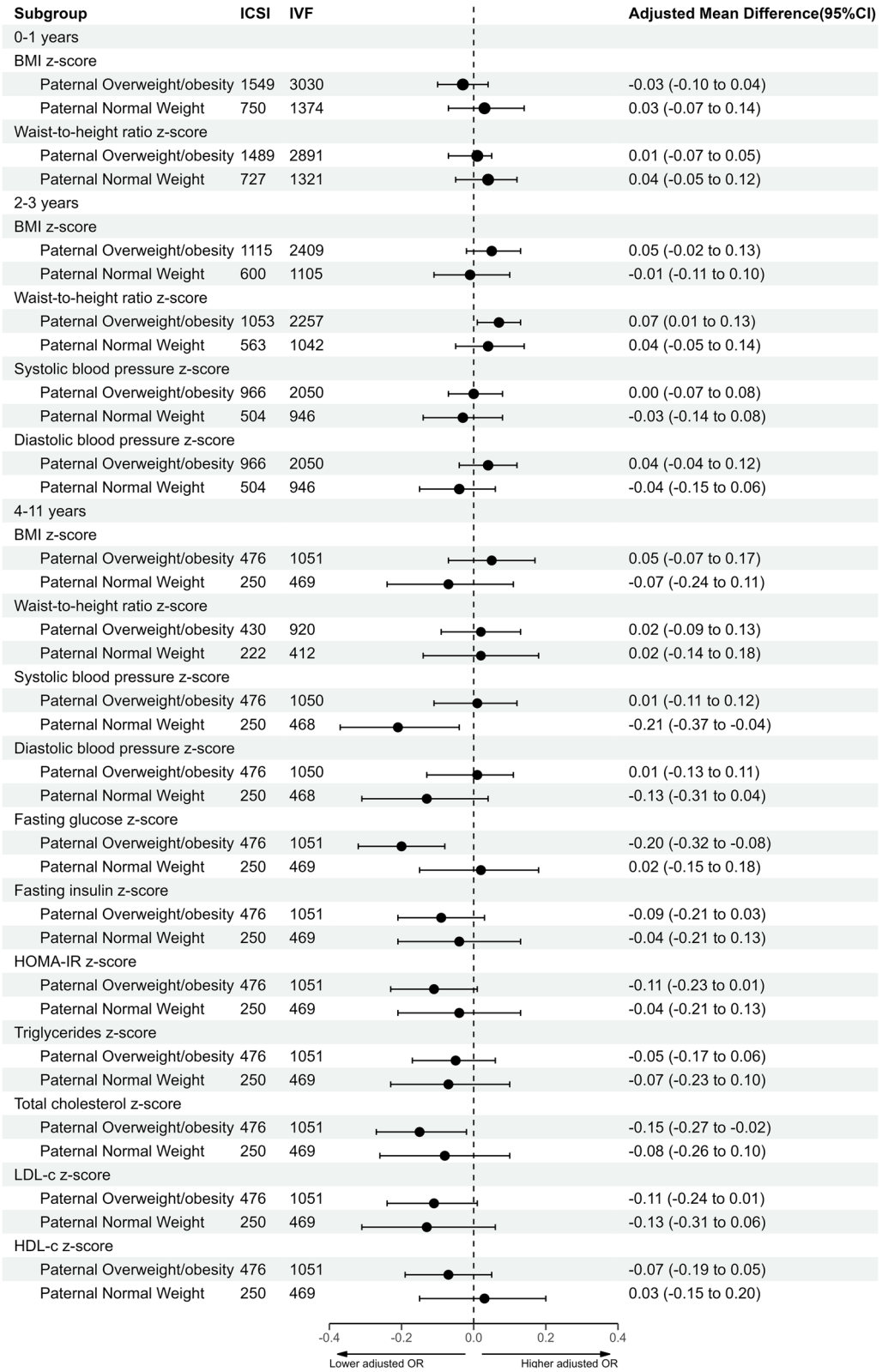

Fig. 2 Associations of paternal overweight/obesity, ICSI treatment, and offspring metabolic alterations

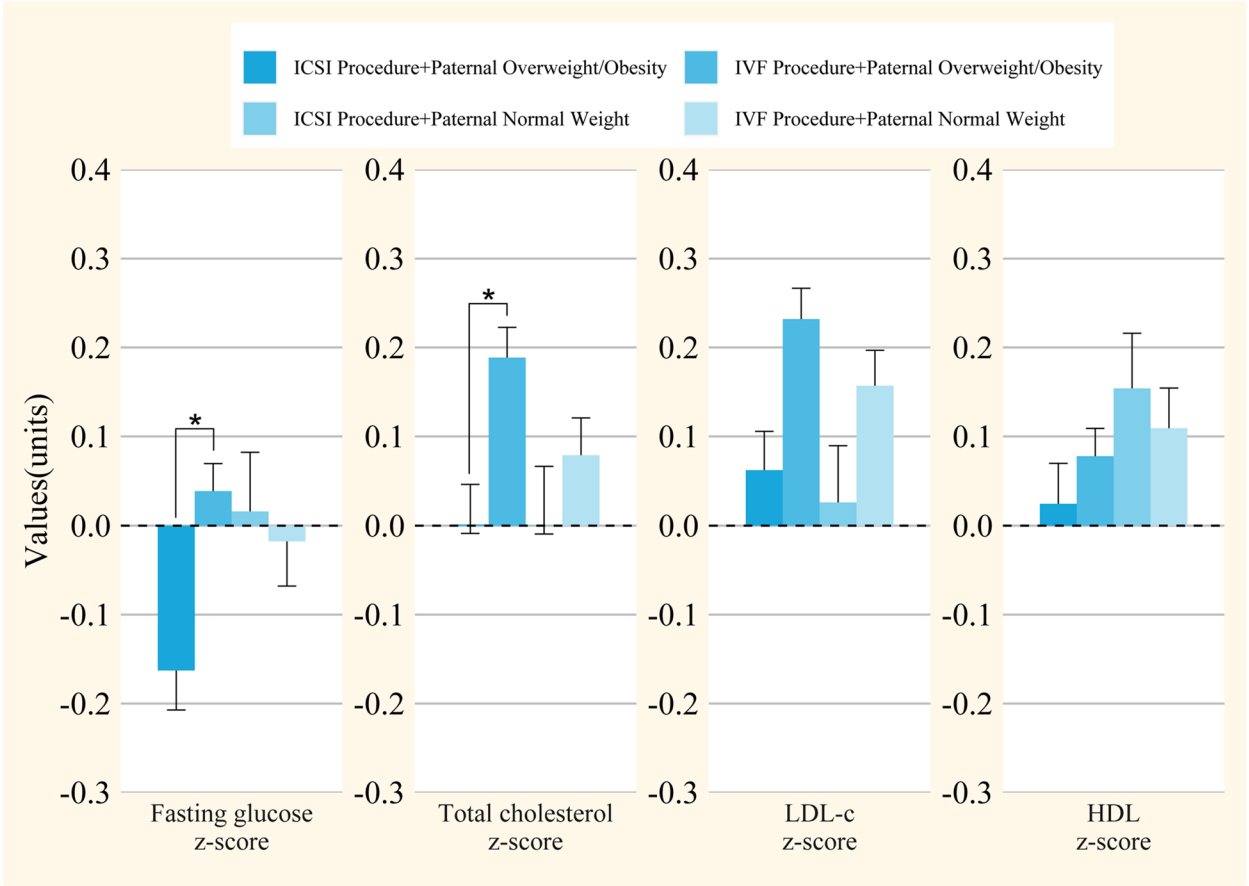

Fig. 3 Associations of paternal overweight/obesity, ICSI, and metabolic alterations in male offspring aged 4–11 years

conceived via ICSI exhibited lower fasting glucose *z*-scores, total cholesterol *z*-scores, and LDL-C *z*-scores compared with their IVF counterparts. Among offspring born to fathers with normal weight, offspring conceived via ICSI displayed a lower systolic blood pressure *z*-score compared with their IVF controls.

Most of the researches demonstrated ICSI is associated with neurodevelopment disorders in children [24–26], while its metabolic effects are few considered. Our study was similar to a previous study, which reported a lower fasting glucose in 121 ICSI-conceived singleton adult men compared with 74 IVF-conceived singleton controls [14]. The clinical significance of low fasting glucose levels was unclear, but there were some clues suggesting a potential association between hypoglycemia and neurodevelopment. Hypoglycemia is the most commonly associated form of dysglycemia with neuronal insult, which can interfere with brain structure development and cognition, leading to deficits in intelligence quotient, learning and memory anomalies, and variations in executive functions [27–30]. In children with type 1 diabetes (T1D), severe hypoglycemia (SH) is in relationship with permanently disrupt cognitive function cognition [31, 32]. The biochemical mechanisms by which hypoglycemia causes neuronal damage are not fully understood. Previous study have reported that hypoglycemia may compromise normal hippocampal development, as evidenced by findings of gliosis and reactive neurogenesis [33]. Additionally, severe hypoglycemia may result in overstimulation of N-methyl-D-aspartate receptors, leading to excitotoxicity and subsequent cell damage [34].

Our study further found a relatively lower total cholesterol and LDL-C level in ICSI-conceived children, consistent with a previous research [14]. However, in our study, these results remained consistent even after adjusting for paternal BMI, paternal age, maternal BMI, maternal age, parity, plurality, parental smoking, frozen embryo transfer, and offspring age. The other study found that ICSI-conceived men had lower mean HDL-C concentrations in comparison to controls spontaneously conceived peers [35]. Reducing cholesterol is widely recognized for its role in protecting against heart disease, but it also rises potential risks to the nervous system [36]. The brain contains approximately 20% of the body's total

cholesterol, making it with the most cholesterol-rich organ in the human body [37]. Cholesterol is essential for the formation and maturation of synapses, playing a critical role in regulating signal transduction as a vital component of cell membranes [38]. It was reported that a low maternal LDL-C level (≤60 mg/dL) was associated with an increased risk of ADHD [39]. The other study found that low cholesterol levels in children were associated with impulsivity in young adulthood [40]. A previous meta-analysis of 19 studies found that IVF-ICSI offspring displayed lower low-density lipoprotein cholesterol levels compared with the metabolism of naturally conceived offspring [41]. Another study found that certain genes involved in cholesterol metabolism were differentially methylated in ICSI offspring compared to naturally conceived children, such as ATG4C and BAZ2B [42]. These epigenetic changes may potentially influence cholesterol metabolism and related health outcomes [42]. Compared with a recent Dutch Lifelines cohort study [43], we found that the total cholesterol and LDL-C levels in IVF-conceived offspring in our data were nearly at the 50th percentile. However, the total cholesterol and LDL-C levels in ICSI-conceived offspring were lower than the 50th percentile but higher than the 10th percentile. We obtained similar results when compared to another study with a relatively longer publication history [44]. One of the meta-analyses published through 2015 demonstrated an overall significant association between depression and low serum LDL-C levels (mean difference: −9.65 mg/dL, 95% CI: −13.81, −5.50, $P<0.001$) in men only studies [45]. Considering the gradually increasing gap in total cholesterol and LDL-C levels between ICSI- and IVF-conceived children, the lower lipid profile of ICSI-conceived offspring might explain the association with potential risks to the nervous system. Overall, while the lower fasting glucose and cholesterol levels of ICSI-conceived children may be associated with a lower risk of cardiovascular diseases, they may also suggest a higher risk of poor neurodevelopment.

The underlying mechanism for ICSI and altered metabolic profile remained unknown. However, previous studies have reported that it is ICSI treatment, not male infertility, associated with an increased risk of disorders in offspring [26]. The potential techniques that involved between ICSI and offspring disorders might include the directly injection of the sperm head and bypass of the natural spermatozoa selection [46]. In rodent models, ICSI treatment could alter the calcium oscillation, which further decreased pre-implantation developmental rates compared with IVF [47]. Importantly, ICSI not only bypasses the fusion of gametes, but also bypasses a series of signaling events that take place before and during conventional sperm–egg interactions [48, 49]. The bypass of these natural barriers could significantly increase the likelihood of using DNA-fragmented spermatozoa (DFS), which may appear morphologically normal and inadvertently be used for ICSI treatment [50]. Elevated levels of DNA damage can lead to embryo arrest and trigger the activation of the apoptotic pathway [51]. Previous studies have reported that the DFS-ICSI may delay male pronucleus demethylation and affect gene transcription and methylation of epigenetically regulated genes, including imprinting, X-linked genes, and retrotransposon genes. These early alterations may result in aberrant growth, premature aging, abnormal behavior, and mesenchymal tumors in the later life their offspring [52].

It was interesting that our study found paternal BMI status, instead of maternal BMI (data not shown), significantly mediated the association between ICSI procedures and offspring metabolic changes. Several studies have reported that, besides specific ART, parental subfertility per se was an important factor associating with metabolic changes in offspring [15]. Primary animal and human studies have reported that parental obesity is casually associated with adverse metabolic alterations in their offspring [17, 53, 54]. It was reported that obese men had higher levels of oxidative stress (OS) and sperm DNA fragmentation compared to normal weight or overweight men [55–57]. This result indicated that the altered metabolic changes in offspring were primarily originated from fathers, and weight loss might be a solution for the metabolic changes in ICSI-conceived children.

Our study had several strengths. The main strength of this study was the large sample size with detailed data collection. The hospital-based cohort we used in this study was the largest ART birth cohort of Chinese Han children. Our study included detailed and accurate data statistics that enhanced the results' statistical validity on this phenomenon. Secondly, the subgroup analysis took into account two risk factors, paternal overweight/obesity and ICSI procedure, which provide a clear method to identify the risks of offspring metabolic disorders in infertile couples undergoing ICSI procedure. Nonetheless, some limitations should also be considered. Firstly, the study population was recruited from a single center, which might not be representative of the general population, and the exclusion due to incomplete anthropometric information and the non-participation of some families could introduce selection bias into the study. Secondly, to minimize the impact of temporal variations and the broad age range of the children, we attempted to control for offspring age in our adjusted model analysis, thereby providing more robust results. Thirdly, the study only followed children up to 11 years of age, which might not capture long-term metabolic effects. Further studies

with longer follow-up periods are needed to assess the persistence of metabolic alterations.

Conclusions

Our study found that ICSI treatment was associated with metabolic changes in male offspring, including lower fasting glucose, total cholesterol, and LDL-C levels. Paternal overweight/obesity significantly mediated these associations. Offspring born to overweight/obese fathers via ICSI showed lower fasting glucose, total cholesterol, and LDL-C levels compared to those born via IVF. Offspring born to fathers with normal weight via ICSI displayed lower systolic blood pressure compared to IVF-conceived offspring. This study addresses a gap in current research by examining the metabolic consequences of ICSI, an area that has received less attention compared to its neurodevelopmental effects.

Abbreviations

ICSI	Intracytoplasmic sperm injection
BMI	Body mass index
CI	Confidence intervals
LDL-C	Low-density lipoprotein cholesterol
IVF	In vitro fertilization
HDL-C	High-density lipoprotein cholesterol
ART	Assisted reproductive technology
ICMART	International Committee for Monitoring Assisted Reproductive Technologies
ASD	Autism spectrum disorder
ADHD	Attention-deficit/hyperactivity disorder
IRB	Institutional review board
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
IQR	Interquartile range
HOMA-IR	Homeostatic model assessment for insulin resistance
SC	Spontaneous conception
T1D	Type 1 diabetes
SH	Severe hypoglycemia
DFS	DNA-fragmented spermatozoa
OS	Oxidative stress

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-024-03654-y.

Additional file 1: Fig. S1. Flowchart of the participant enrolment in this study. *N*: number of families. *n*: number of offspring visits. *A total of 15,415 offspring with 27,698 visits were identified. †7816 male offspring with 14,196 visits were included.

Additional file 2: Table S1. Characteristics of male offspring stratified by paternal BMI status.

Additional file 3: Figure S2, Tables S2–S6. Fig. S2. Associations between ICSI treatment and offspring metabolic alterations in male singletons. Table S2. Characteristics of study offspring. Table S3. Associations between ICSI treatment and offspring metabolic alterations. Table S4. The age-specific total cholesterol, LDL-C, and HDL-C levels in ICSI- and IVF-conceived children. Table S5. Characteristics of offspring in male singleton. Table S6. Differences between ICSI-conceived and IVF-conceived singleton in male offspring.

Additional file 4: Figure S3, Tables S7–S10. Fig. S3. Associations of paternal overweight/obesity, ICSI treatment, and offspring metabolic alterations in male singletons. Table S7. Characteristics of male offspring stratified by paternal BMI status and ICSI methods. Table S8. Associations of paternal obesity, ICSI treatment, and offspring metabolic alterations. Table S9. Characteristics of offspring stratified by paternal BMI status and ICSI treatment in male singletons. Table S10. Differences between ICSI-conceived singleton and IVF-conceived singleton in male offspring.

Additional file 5: Tables S11–S12. Table S11. Characteristics of offspring stratified by ICSI methods. Table S12. Associations of paternal obesity, PESA/TES-ICSI, and male offspring metabolic alterations.

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Authors' contributions

BZ designed the study. MB, XC, and JH were involved in data acquisition. BZ and MB conducted the clinical data analysis. BZ drafted the manuscript, and LC and ZJC reviewed and gave final approval of the version to be published. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

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Data availability

The data underlying this article were provided by Center for Reproductive Medicine, Cheeloo College of Medicine, Shandong University, under license/by permission. Anonymized data could be shared on reasonable request to the corresponding author, Linlin Cui (liy@sdu.edu.cn), with permission of Center for Reproductive Medicine, Cheeloo College of Medicine, Shandong University.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board (IRB) of the Children and Reproductive Health Institute of Women, Shandong University (Ethics Approval Number: [2014] Lun Shen Zi (17) No.). Written informed consent was obtained from the parents for their participation and that of their offspring.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Children and Reproductive Health, Institute of Women, Jinan, Shandong 250012, China. [2]The Second Hospital, Children and Reproductive Health, Institute of Women, Shandong University, Jinan, Shandong 250012, China. [3]School of Basic Medical Sciences, Shandong University, Jinan, Shandong 250012, China. [4]State Key Laboratory of Reproductive Medicine and Offspring Health, Shandong University, Jinan, Shandong 250012, China. [5]National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Shandong University, Jinan, Shandong 250012, China. [6]Key Laboratory of Reproductive Endocrinology (Shandong University), Ministry of Education, Jinan, Shandong 250012, China. [7]Shandong Technology Innovation Center for Reproductive Health, Jinan, Shandong 250012, China. [8]Shandong Provincial Clinical Research Center for Reproductive Health, Jinan, Shandong 250012, China. [9]Shandong Key Laboratory of Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong First Medical University, Jinan 250012, China. [10]ResearchUnit of Gametogenesis and Health of ART-Offspring, Chinese Academy of Medical Sciences (No. 2021RU001), Jinan, Shandong 250012, China. [11]Department of Reproductive Medicine, Ren Ji Hospital, Shanghai Jiao

Tong University School of Medicine, Shanghai 200135, China. [12]Shanghai Key Laboratory for Assisted Reproduction and Reproductive Genetics, Shanghai 200135, China. [13]Department of Obstetrics and Gynecology, The First Affiliated Hospital of Shandong First Medical University, Jinan 250012, China.

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References

1. Wang C, Lv H, Ling X, Li H, Diao F, Dai J, Du J, Chen T, Xi Q, Zhao Y, et al. Association of assisted reproductive technology, germline de novo mutations and congenital heart defects in a prospective birth cohort study. Cell Res. 2021;31(8):919–28.
2. Zhang B, Wang Z, Dai K, Cui L, Chen ZJ. Associations of maternal obesity, frozen embryos, and offspring adverse cardiometabolic alterations. Fertil Steril. 2022;118(6):1117–26.
3. Weng SS, Huang YT, Huang YT, Li YP, Chien LY. Assisted reproductive technology and risk of childhood cancers. JAMA Netw Open. 2022;5(8): e2230157.
4. Tannus S, Son WY, Gilman A, Younes G, Shavit T, Dahan MH. The role of intracytoplasmic sperm injection in non-male factor infertility in advanced maternal age. Hum Reprod. 2017;32(1):119–24.
5. Esteves SC, Roque M, Bedoschi G, Haahr T, Humaidan P. Intracytoplasmic sperm injection for male infertility and consequences for offspring. Nat Rev Urol. 2018;15(9):535–62.
6. O'Neill CL, Chow S, Rosenwaks Z, Palermo GD. Development of ICSI. Reproduction. 2018;156(1):F51-f58.
7. de Mouzon J, Chambers GM, Zegers-Hochschild F, Mansour R, Ishihara O, Banker M, Dyer S, Kupka M, Adamson GD. International Committee for Monitoring Assisted Reproductive Technologies world report: assisted reproductive technology 2012†. Hum Reprod. 2020;35(8):1900–13.
8. Zheng MM, Cao HR, Zhang WY, Yan PP, Xu JY, Zhao HL, Zhu F, Zhang JJ, Li Y, Zhu H. Abnormal gene methylation during embryonic development after preimplantation genetic testing increases risk of liver-derived insulin resistance. Ann N Y Acad Sci. 2018;1425(1):70–81.
9. Zhu Z, Cao F, Li X. Epigenetic programming and fetal metabolic programming. Front Endocrinol (Lausanne). 2019;10:764.
10. Kurinczuk JJ. Safety issues in assisted reproduction technology. From theory to reality—just what are the data telling us about ICSI offspring health and future fertility and should we be concerned? Hum Reprod. 2003;18(5):925–31.
11. Belva F, Bonduelle M, Roelants M, Michielsen D, Van Steirteghem A, Verheyen G, Tournaye H. Semen quality of young adult ICSI offspring: the first results. Hum Reprod. 2016;31(12):2811–20.
12. Jagadapillai R, Singh K. Editorial: metabolic traits associated with neurodevelopmental and neuropsychiatric disorders. Front Genet. 2023;14: 1319341.
13. Hoirisch-Clapauch S, Nardi AE. Autism spectrum disorders: let's talk about glucose? Transl Psychiatry. 2019;9(1):51.
14. Catford SR, Halliday J, Lewis S, O'Bryan MK, Handelsman DJ, Hart RJ, McBain J, Rombauts L, Amor DJ, Saffery R, et al. The metabolic health of young men conceived using intracytoplasmic sperm injection. Hum Reprod. 2022;37(12):2908–20.
15. Berntsen S, Söderström-Anttila V, Wennerholm UB, Laivuori H, Loft A, Oldereid NB, Romundstad LB, Bergh C, Pinborg A. The health of children conceived by ART: 'the chicken or the egg?' Hum Reprod Update. 2019;25(2):137–58.
16. Godfrey KM, Reynolds RM, Prescott SL, Nyirenda M, Jaddoe VW, Eriksson JG, Broekman BF. Influence of maternal obesity on the long-term health of offspring. Lancet Diab Endocrinol. 2017;5(1):53–64.
17. Sharp GC, Lawlor DA. Paternal impact on the life course development of obesity and type 2 diabetes in the offspring. Diabetologia. 2019;62(10):1802–10.
18. Fleming TP, Watkins AJ, Velazquez MA, Mathers JC, Prentice AM, Stephenson J, Barker M, Saffery R, Yajnik CS, Eckert JJ, et al. Origins of lifetime health around the time of conception: causes and consequences. Lancet. 2018;391(10132):1842–52.
19. Zhou BF. Predictive values of body mass index and waist circumference for risk factors of certain related diseases in Chinese adults—study on optimal cut-off points of body mass index and waist circumference in Chinese adults. Biomed Environ Sci. 2002;15(1):83–96.
20. Chen W. Guidelines for medical nutrition treatment of overweight/obesity in China (2021). Asia Pac J Clin Nutr. 2022;31(3):450–82.
21. Sun J, Hu J, Zhou X, Li J, Hu K, Sun Y, Cao F, Cui L, Chen ZJ. Relationship between anxiety and depressive trajectories of women who conceived through assisted reproductive technology and their children's emotional and behavioral problems: a prospective cohort study. J Affect Disord. 2023;332:150–8.
22. Cui L, Zhou W, Xi B, Ma J, Hu J, Fang M, Hu K, Qin Y, You L, Cao Y, et al. Increased risk of metabolic dysfunction in children conceived by assisted reproductive technology. Diabetologia. 2020;63(10):2150–7.
23. Fu J, Li Y, Esangbedo IC, Li G, Feng D, Li L, Xu L, Han L, Li M, Li C, et al. Circulating osteonectin and adipokine profiles in relation to metabolically healthy obesity in Chinese children: findings from BCAMS. J Am Heart Assoc. 2018;7(23): e009169.
24. Bowen JR, Gibson FL, Leslie GI, Saunders DM. Medical and developmental outcome at 1 year for children conceived by intracytoplasmic sperm injection. Lancet. 1998;351(9115):1529–34.
25. Knoester M, Helmerhorst FM, Vandenbroucke JP, van der Westerlaken LA, Walther FJ, Veen S. Cognitive development of singletons born after intracytoplasmic sperm injection compared with in vitro fertilization and natural conception. Fertil Steril. 2008;90(2):289–96.
26. Lo H, Weng SF, Tsai EM. Neurodevelopmental disorders in offspring conceived via in vitro fertilization vs intracytoplasmic sperm injection. JAMA Netw Open. 2022;5(12): e2248141.
27. Cacciatore M, Grasso EA, Tripodi R, Chiarelli F. Impact of glucose metabolism on the developing brain. Front Endocrinol (Lausanne). 2022;13:1047545.
28. Lin A, Northam EA, Rankins D, Werther GA, Cameron FJ. Neuropsychological profiles of young people with type 1 diabetes 12 yr after disease onset. Pediatr Diab. 2010;11(4):235–43.
29. Perantie DC, Lim A, Wu J, Weaver P, Warren SL, Sadler M, White NH, Hershey T. Effects of prior hypoglycemia and hyperglycemia on cognition in children with type 1 diabetes mellitus. Pediatr Diab. 2008;9(2):87–95.
30. Asvold BO, Sand T, Hestad K, Bjørgaas MR. Cognitive function in type 1 diabetic adults with early exposure to severe hypoglycemia: a 16-year follow-up study. Diab Care. 2010;33(9):1945–7.
31. Ryan CM, Becker DJ. Hypoglycemia in children with type 1 diabetes mellitus. Risk factors, cognitive function, and management. Endocrinol Metab Clin North Am. 1999;28(4):883–900.
32. Lacy ME, Gilsanz P, Eng C, Beeri MS, Karter AJ, Whitmer RA. Severe hypoglycemia and cognitive function in older adults with type 1 diabetes: the Study of Longevity in Diabetes (SOLID). Diab Care. 2020;43(3):541–8.
33. Hershey T, Perantie DC, Wu J, Weaver PM, Black KJ, White NH. Hippocampal volumes in youth with type 1 diabetes. Diabetes. 2010;59(1):236–41.
34. Arbelaez AM, Semenkovich K, Hershey T. Glycemic extremes in youth with T1DM: the structural and functional integrity of the developing brain. Pediatr Diabetes. 2013;14(8):541–53.
35. Belva F, Bonduelle M, Provyn S, Painter RC, Tournaye H, Roelants M, De Schepper J. Metabolic syndrome and its components in young adults conceived by ICSI. Int J Endocrinol. 2018;2018:8170518.
36. Martín MG, Pfrieger F, Dotti CG. Cholesterol in brain disease: sometimes determinant and frequently implicated. EMBO Rep. 2014;15(10):1036–52.
37. Mahley RW. Central nervous system lipoproteins: ApoE and regulation of cholesterol metabolism. Arterioscler Thromb Vasc Biol. 2016;36(7):1305–15.
38. Pfrieger FW. Role of cholesterol in synapse formation and function. Biochim Biophys Acta. 2003;1610(2):271–80.
39. Ji Y, Riley AW, Lee LC, Volk H, Hong X, Wang G, Angomas R, Stivers T, Wahl A, Ji H, et al. A prospective birth cohort study on maternal cholesterol levels and offspring attention deficit hyperactivity disorder: new insight on sex differences. Brain Sci. 2017;8(1):3.
40. Tomson-Johanson K, Kaart T, Kiivet RA, Veidebaum T, Harro J. Low cholesterol levels in children predict impulsivity in young adulthood. Acta neuropsychiatrica. 2020;32(4):196–205.
41. Guo XY, Liu XM, Jin L, Wang TT, Ullah K, Sheng JZ, Huang HF. Cardiovascular and metabolic profiles of offspring conceived by assisted reproductive technologies: a systematic review and meta-analysis. Fertil Steril. 2017;107(3):622-631.e625.

42. El Hajj N, Haertle L, Dittrich M, Denk S, Lehnen H, Hahn T, Schorsch M, Haaf T. DNA methylation signatures in cord blood of ICSI children. Hum Reprod. 2017;32(8):1761–9.
43. Balder JW, Lansberg PJ, Hof MH, Wiegman A, Hutten BA, Kuivenhoven JA. Pediatric lipid reference values in the general population: the Dutch lifelines cohort study. J Clin Lipidol. 2018;12(5):1208–16.
44. Can M, Piskin E, Guven B, Acikgoz S, Mungan G. Evaluation of serum lipid levels in children. Pediatr Cardiol. 2013;34(3):566–9.
45. Persons JE, Fiedorowicz JG. Depression and serum low-density lipoprotein: a systematic review and meta-analysis. J Affect Disord. 2016;206:55–67.
46. Sánchez-Calabuig MJ, López-Cardona AP, Fernández-González R, Ramos-Ibeas P, Fonseca Balvís N, Laguna-Barraza R, Pericuesta E, Gutiérrez-Adán A, Bermejo-Álvarez P. Potential health risks associated to ICSI: insights from animal models and strategies for a safe procedure. Front Public Health. 2014;2:241.
47. Kurokawa M, Fissore RA. ICSI-generated mouse zygotes exhibit altered calcium oscillations, inositol 1,4,5-trisphosphate receptor-1 down-regulation, and embryo development. Mol Hum Reprod. 2003;9(9):523–33.
48. Shirazi A, Ostad-Hosseini S, Ahmadi E, Heidari B, Shams-Esfandabadi N. In vitro developmental competence of ICSI-derived activated ovine embryos. Theriogenology. 2009;71(2):342–8.
49. Ohlweiler LU, Brum DS, Leivas FG, Moyses AB, Ramos RS, Klein N, Mezzalira JC, Mezzalira A. Intracytoplasmic sperm injection improves in vitro embryo production from poor quality bovine oocytes. Theriogenology. 2013;79(5):778–83.
50. Zini A, Meriano J, Kader K, Jarvi K, Laskin CA, Cadesky K. Potential adverse effect of sperm DNA damage on embryo quality after ICSI. Hum Reprod. 2005;20(12):3476–80.
51. Alvarez Sedó C, Bilinski M, Lorenzi D, Uriondo H, Noblía F, Longobucco V, Lagar EV, Nodar F. Effect of sperm DNA fragmentation on embryo development: clinical and biological aspects. JBRA Assist Reprod. 2017;21(4):343–50.
52. Fernández-Gonzalez R, Moreira PN, Pérez-Crespo M, Sánchez-Martín M, Ramirez MA, Pericuesta E, Bilbao A, Bermejo-Alvarez P, de Dios HJ, de Fonseca FR, 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(4):761–72.
53. McPherson NO, Fullston T, Aitken RJ, Lane M. Paternal obesity, interventions, and mechanistic pathways to impaired health in offspring. Ann Nutr Metab. 2014;64(3–4):231–8.
54. Oldereid NB, Wennerholm UB, Pinborg A, Loft A, Laivuori H, Petzold M, Romundstad LB, Söderström-Anttila V, Bergh C. The effect of paternal factors on perinatal and paediatric outcomes: a systematic review and meta-analysis. Hum Reprod Update. 2018;24(3):320–89.
55. Fariello RM, Pariz JR, Spaine DM, Cedenho AP, Bertolla RP, Fraietta R. Association between obesity and alteration of sperm DNA integrity and mitochondrial activity. BJU Int. 2012;110(6):863–7.
56. Yang Q, Zhao F, Hu L, Bai R, Zhang N, Yao G, Sun Y. Effect of paternal overweight or obesity on IVF treatment outcomes and the possible mechanisms involved. Sci Rep. 2016;6:29787.
57. Pearce KL, Hill A, Tremellen KP. Obesity related metabolic endotoxemia is associated with oxidative stress and impaired sperm DNA integrity. Basic Clin Androl. 2019;29:6.

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