



## Serum levels of trace elements in children born after assisted reproductive technology

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### ABSTRACT

**Introduction:** The health and development of newborn children born via assisted reproductive technology (ART), as well as their health in adulthood, have raised great concern. This study was designed to investigate whether ART children have differences in the levels of trace elements compared with naturally conceived children.

**Methods:** This study included those ART children and controls aged 1 to 12 years assessed with a follow-up protocol. Serum levels of the trace elements zinc, copper, iron, calcium, magnesium and lead were determined and analyzed.

**Results:** There were no significant differences in age, gender or body weight between the ART and control groups. There were no significant differences in the rates of deficiency or excess of trace elements between the two groups. Serum lead levels in children born via ART were significantly higher than those in the controls, whereas the levels of zinc and iron were significantly decreased in the ART group, although these levels were still within the normal ranges.

**Discussion:** These results indicate the need to monitor the blood levels of zinc, iron and lead in ART children aged 1–6 years old. These findings contribute to our understanding on the long-term safety of ART and may facilitate screening for potential diseases related to trace elements.

### 1. Introduction

Assisted reproductive technologies (ARTs), such as artificial insemination (AI), in vitro fertilization and embryo transfer (IVF-ET), intracytoplasmic sperm injection (ICSI), frozen-thawed embryo transfer (FET) and preimplantation genetic diagnosis (PGD), have been widely used to resolve human infertility. Currently, > 5 million babies worldwide have been born via in vitro fertilization alone [1]. The global use of ART has led to a major concern about its safety, especially regarding pregnancy outcomes and the health of newborn children. In

the last few years, many studies have been conducted in this field [2]. It has been reported that ART is associated with intrauterine growth restriction (IUGR) and congenital malformations [3–5]. Hansen et al. [6] reported that infants conceived by IVF or ICSI had a two-fold higher risk for major birth defects than naturally conceived infants. Increased rates of multiple pregnancies, low birth weight (LBW; < 2500 g), very low birth weight (VLBW; < 1500 g) and low gestational age (< 37 weeks gestation) have also been observed [2,7–13]. Additionally, some studies have suggested that compared with spontaneously conceived children, ART children have increased risks of epigenetic and imprinting

**Abbreviations:** AI, artificial insemination; AIH, artificial insemination by husband; ANOVA, analysis of variance; ART, assisted reproductive technology; As, arsenic; Ca, calcium; CCRM, Center of Clinical Reproductive Medicine; Cd, cadmium; Cl, chlorine; Co, cobalt; Cr, chromium; Cu, copper; Ems, endometriosis; F, fluorine; Fe, iron; FET, frozen thawed embryo transfer; Hg, mercury; I, iodine; ICSI, intracytoplasmic sperm injection; ID, iron deficiency; IUGR, intrauterine growth retardation; IVF-ET, in vitro fertilization and embryo transfer; K, potassium; LBW, low birth weight; Mg, magnesium; Mn, manganese; Mo, molybdenum; Na, sodium; Ni, nickel; P, phosphorus; Pb, lead; PCOS, polycystic ovary syndrome; PGD, preimplantation genetic diagnosis; SD, standard deviation; Se, selenium; V, vanadium; VLBW, very low birth weight; Zn, zinc

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disorders, neurologic sequelae (low learning ability and language barrier) [5,14–17], and specific abnormalities, such as unspecified infection and parasitic diseases, asthma, genitourinary diseases, and epilepsy or convulsions [18,19]. The increased prevalence of LBW or VLBW, prematurity, and IUGR in ART children may place them at high risk of metabolic syndrome (obesity, type 2 diabetes and hypertension) in adulthood [5]. Ceelen et al. [20] found that blood pressure was higher in IVF-conceived children compared with naturally conceived children aged 8- to 18-year-old. Our previous study found that the ultrastructure and gene expression profile of ART-derived placentas were different from naturally derived placentas, which may potentially affect the health of the offspring [21]. The mechanisms underlying the adverse health risks of offspring conceived by ART remain unclear, and whether the potentially increased health risk is caused by ART or other factors associated with infertility remains inconclusive.

Recently, the impact of micronutrients on fetal health has become a hot topic. Micronutrients are essential nutrients needed for life. There are four main types of micronutrients: macro-elements, trace elements, vitamins, and organic acids. Trace elements, including zinc (Zn), copper (Cu), selenium (Se), iodine (I), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), fluorine (F), and molybdenum (Mo), are dietary minerals that are required for the normal growth, development and physiological function of an organism. The content of trace elements is < 0.01% of body weight, and the daily requirement is < 100 mg in total [22]. The importance of trace elements cannot be ignored, as they participate in tissue, cellular and subcellular processes, including nerve conduction, muscle contraction, membrane potential regulation, mitochondrial activity, and the enzyme-mediated regulation of humoral and cellular immunity [23].

The shortage or excess of certain element(s) can affect human health or lead to disease. For example, iron deficiency is a common disease with adverse effects on physical and psychological development during pregnancy and childhood [22,24]. In type 2 diabetes, Fe and V levels are increased, whereas Ca, Mg, Na, Cr, Co, I, Fe, Se, Mn, and Zn levels tend to be decreased [23]. Meanwhile, certain trace elements, such as arsenic (As), cadmium (Cd), mercury (Hg), nickel (Ni) and lead (Pb), represent potential hazards as exposure to these elements lead to toxic or carcinogenic effects and/or oxidative stress [25,26].

This study was designed to investigate whether there is a difference in blood levels of six trace elements between ART and naturally conceived children and whether there is a correlation between the levels of trace elements and the long-term health of ART children.

## 2. Materials and methods

### 2.1. Study population

Children conceived by ART (ART group) and children conceived naturally (control group) in Jiangsu Province from 2012 to 2015 were included in this study. The ART-conceived offspring included live-born infants from 2001 to 2014 in the Clinic Center of Reproductive Medicine of Jiangsu Province Hospital. The two groups were evenly distributed in Jiangsu Province and surrounding area in China. During the research period from 2001 to 2015, there was no report of major air pollution, water pollution or food safety incidents in this area. Except for 11 families whose fathers or mothers were the Hui nationality living in Han settle area (Jiangsu Province), all of other parents were Han nationality of China. The dietary review of the two groups showed a balanced diet, without dietary preference. Therefore, there were not significant differences in the race, region, climate, nutrition, dietary habit, education, and the ratio of urban and rural population between among groups. The informed consent was obtained from a guardian of each participant included in this study. All data were derived from the Center of Clinical Reproductive Medicine (CCRM) Management System database. The children were grouped by age, as follows: the 1–3 year-old, 3–6 year-old, and 6–12 year-old groups. Distribution of different

**Table 1**  
Distribution of study subjects by ART methods and age.

ART method	1–3 years old group (n = 197)	3–6 years old group (n = 186)	6–12 years old group (n = 32)
AIH <sup>a</sup>	11	9	1
IVF-ET <sup>b</sup>	132	108	25
ICSI <sup>c</sup>	52	64	5
Others	2	4	1

Others: including PGD (preimplantation genetic diagnosis) and donated egg.

<sup>a</sup> AIH, artificial insemination by husband.

<sup>b</sup> IVF-ET, in vitro fertilization and embryo transfer.

<sup>c</sup> ICSI, intracytoplasmic sperm injection.

**Table 2**  
Distribution of study subjects by infertility factors and age.

Infertility factor	1–3 years old group (n = 197)	3–6 years old group (n = 186)	6–12 years old group (n = 32)
Paternal factors			
Oligoasthenoteratozoospermia or azoospermia <sup>a</sup>	56	63	4
Maternal factors			
PCOS or Ovulation failure or hypo-ovaria <sup>b</sup>	45	14	2
EMs <sup>c</sup>	3	10	4
Oviducal and pelvic disease <sup>d</sup>	34	58	11
a + b/c	25	10	0
d + a/b	27	16	5
Others	6	14	5
Missing values	1	1	1

Others: unknown reasons or other reasons.

<sup>a</sup> Oligoasthenoteratozoospermia or azoospermia.

<sup>b</sup> PCOS, polycystic ovary syndrome.

<sup>c</sup> EMs, endometriosis.

<sup>d</sup> Oviducal and pelvic disease.

ART methods in ART-conceived children was shown in Table 1. Distribution of different infertility factors in ART-conceived children was shown in Table 2. The control children were recruited from healthy volunteers undergoing physical examination. The two groups were matched according to their age in months. Children with abnormal physical examination results, metabolic disorders or chromosomal abnormalities were excluded. Families with incomplete data records or difficult to follow-up with were also excluded. For families with multiple births, we chose only the first child to eliminate the effects of multiple births. The final numbers of ART and control children were 415 and 419, respectively.

### 2.2. Serum collection and detection

The children were asked to wash their hands before being examined by professional personnel, and their ring fingers were sterilized with 75% medical alcohol. After volatilization, disposable capillaries were used to acquire 80 µL of peripheral blood, which was then divided into two aliquots. One was for measurements of Pb using a graphite furnace, another was for Zn, Cu, Fe, Ca and Mg measurements using a flame and they were placed in special reagent tubes for the measurements. The levels of peripheral blood Zn, Cu, Fe, Ca, Mg and Pb were analyzed by a AA7000M atomic absorption spectrometer (Beijing East & West Analytical Instruments Co., Ltd.). The standard sample offered by the company was used for quality control. The concentration measurement process was as follows: booting, preheating, standard calibration, sample placement into the machine, and automatic output of the results. The detection results were within the standard reference range, below the lower limit of the normal range (element deficiency), or above the upper limit (excess of the element). The following normal

ranges for different elements were applied: Zn (mg/L): 5–11.94; Cu (mg/L): 0.76–2.5; Fe (mg/L): 418.48–660.8; Ca (mg/L): 84–62.86; Mg (mg/L): 28.3–50.4; and Pb( $\mu$ g/L): 0–100.

### 2.3. Body length, height and weight

Weight, length or height was measured at the Department of Children's Health Care by skilled personnel. Length was measured with the child fully extended in the supine position. We used a moveable headboard to measure the length with accuracy within 0.1 cm.

### 2.4. Statistical analysis

Data were analyzed using IBM SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). Continuous data are presented as mean  $\pm$  standard deviation (SD). Categorical data are presented as percentages (%). The averages of different groups were compared using an independent *t*-test, and categorical data were compared using the chi square test. Statistical significance was defined as  $p < 0.05$ .

## 3. Results

### 3.1. General baseline characteristics of the participants

To determine the comparability of age, gender, birth and growth status, we evaluated the baseline characteristics of the cohort (Table 3). There was no significant difference in age, gender, length and weight between the ART and control groups among different age groups, except for a significantly reduced length in the 3–6 year-old ART group compared with the control group ( $p = 0.043$ ).

### 3.2. Occurrence of trace element deficiency or excess in the cohort of children

The levels of trace elements in children of different ages are shown in Table 4. In the 1–3 year-old group, Zn deficiency was observed in 18 ART children (9.14%) and 14 non-ART children (7.11%) (no significant difference), whereas iron deficiency was observed in 7 ART children (3.55%) and 11 non-ART children (5.58%) (no significant difference). The contents of Ca, Mg, Cu and Pb in all the studied cases were within the normal ranges.

In the 3–6 year-old group, only one case record showed excess Pb levels (164.60  $\mu$ g/L), and this child was in the ART group. Zn deficiency was observed in 10 ART children (5.38%) and 3 non-ART children (1.58%) ( $p = 0.051$ ), and iron deficiency was observed in 4 ART children (2.15%) and 4 non-ART children (2.11%). The contents of Ca, Mg and Cu were within the normal ranges.

In the 6–12 year-old group, only one case each of Zn and Fe deficiency was observed. Ca, Mg, Cu and Pb levels were within the normal ranges.

Our study showed that there was no significant difference in the

prevalence of abnormal levels of the six trace elements between the ART and naturally conceived children. There was no significant difference in the rates of elemental deficiency or excess between the matched groups across different ages in either ART or control group.

### 3.3. Average level of trace elements in the ART and control groups

The mean trace element levels were all within the normal ranges (Table 5). Although the average level in both groups fell within the normal range, in the 1–3 year-old group, serum Pb was a highly significant increase in children born via ART compared with naturally conceived children (mean  $\pm$  SD, 5.49  $\pm$  0.41 vs 6.23  $\pm$  3.39 mg/L,  $p < 0.001$ ). In contrast, serum Zn was significantly lower in the ART group than in the control group ( $p < 0.01$ ). However, the average levels of both Pb and Zn in both ART and control cohorts in the studied age groups were within the normal range.

In the 3–6 year-old group, serum Pb was significantly higher in children born via ART than in naturally conceived children (mean  $\pm$  SD, 47.83  $\pm$  13.39 vs 39.94  $\pm$  9.59 mg/L,  $p < 0.001$ ). In contrast, the serum Zn and Fe concentrations were significantly lower in the ART group than in the control group ( $p < 0.001$ ). Despite these differences, all these average levels were within the normal ranges.

In the 6–12 year-old group, the Mg and Ca levels were significantly higher in children born via ART than in naturally conceived children ( $p < 0.01$ ).

The distributions of trace elements in the ART and control groups are shown in Fig. 1.

## 4. Discussion

We conducted a health tracking survey of human ART-conceived offspring to assess the levels of six trace elements and compared the results with those of naturally conceived children. We observed differences between ART children and naturally conceived in certain age groups. Despite these differences, none of the average values were outside the normal ranges. Thus, ART is relatively safe and may not be associated with pathological excess or deficiency in the levels of trace elements. These findings seem to suggest that ART is associated with increased levels of trace elements. Since trace elements are critical for normal development and health, as discussed in detail below, we could not exclude the possibility that ART is related to alterations of the levels and usage of trace elements, which may lead to some subclinical manifestation or health risks. Therefore, ART children may be required to receive regular screenings on element levels, and substantial the excess or deficiency, if detected should be taken into consideration during health assessment or diagnosis of related diseases.

Numerous studies have been performed to understand the physiological functions of trace elements as well as their effects on health. During gestation and the early infant stage, deficiencies in nutrients, such as amino acids, vitamins, and trace elements, have irreversible deleterious effects on the development of the limbic system [27]. The

**Table 3**  
General characteristics of subjects (Mean  $\pm$  SD or %).

	Age (month)	Length (cm)	Weight (kg)	Gender	Male	Female
ART group at 1–3 years old (n = 197)	23.78 $\pm$ 7.26	88.10 $\pm$ 8.22	12.65 $\pm$ 2.36		108(54.8%)	89(45.2%)
Control group at 1–3 years old (n = 197)	23.38 $\pm$ 7.88	86.13 $\pm$ 8.38	12.74 $\pm$ 4.56		111(56.3%)	86(43.7%)
P-value	0.654	0.086	0.839	0.839		
ART group at 3–6 years old (n = 186)	47.33 $\pm$ 8.99	105.71 $\pm$ 7.29	17.58 $\pm$ 3.52		99(53.2%)	87(46.8%)
Control group at 3–6 years old (n = 190)	47.24 $\pm$ 11.47	107.69 $\pm$ 7.19	17.79 $\pm$ 2.82		90(47.4%)	100(52.6%)
P-value	0.936	0.043*	0.649	0.259		
ART group at 6–12 years old (n = 32)	83.66 $\pm$ 8.68	126.96 $\pm$ 5.69	26.00 $\pm$ 6.66		19(59.4%)	13(40.6%)
Control group at 6–12 years old (n = 32)	88.42 $\pm$ 15.30	126.72 $\pm$ 6.63	27.14 $\pm$ 6.11		17(53.1%)	15(46.9%)
P-value	0.115	0.878	0.483	0.801		

\*  $P < 0.05$ .

**Table 4**  
Occurrence of trace elements deficiency or excess in the cohort of children.

	Magnesium deficiency	Zinc deficiency	Copper deficiency	Iron deficiency	Calcium deficiency	Lead excess
ART group at 1–3 years old (n = 197)	0	18(9.14%)	0	7(3.55%)	0	0
Control group at 1–3 years old (n = 197)	0	14(7.11%)	0	11(5.58%)	0	0
P-value		0.581		0.470		
ART group at 3–6 years old (n = 186)	0	10(5.38%)	0	4(2.15%)	0	1(0.54%)
Control group at 3–6 years old (n = 190)	0	3(1.58%)	0	4(2.11%)	0	0(0%)
P-value		0.051		0.721		0.495
ART group at 6–12 years old (n = 32)	0	1(3.13%)	0	0(0%)	0	0
Control group at 6–12 years old (n = 32)	0	0(0%)	0	1(3.13%)	0	0
P-value		1.000		1.000		

**Table 5**  
Levels of trace elements in ART and control groups.

	Mg (mg/L)	Zn (mg/L)	Cu (mg/L)	Fe (mg/L)	Ca (mg/L)	Pb (µg/L)
ART group at 1–3 years old (n = 197)	36.76 ± 3.67	5.49 ± 0.41	1.26 ± 0.14	431.72 ± 11.92	66.87 ± 2.18	44.88 ± 10.29
Control group at 1–3 years old (n = 197)	37.10 ± 5.12	6.23 ± 3.39	1.27 ± 0.45	429.05 ± 45.04	67.31 ± 2.71	36.18 ± 9.77
P-value	0.454	0.003**	0.623	0.422	0.101	0.000***
ART group at 3–6 years old (n = 186)	36.50 ± 3.60	5.78 ± 0.59	1.25 ± 0.17	434.05 ± 11.26	67.14 ± 2.29	47.83 ± 13.39
Control group at 3–6 years old (n = 190)	36.79 ± 4.28	6.12 ± 0.70	1.25 ± 0.17	438.91 ± 12.22	66.86 ± 2.31	39.94 ± 9.59
P-value	0.467	0.000***	0.983	0.000***	0.247	0.000***
ART group at 6–12 years old (n = 32)	38.34 ± 4.00	5.88 ± 0.52	1.30 ± 0.20	437.14 ± 17.40	68.33 ± 2.52	46.23 ± 12.27
Control group at 6–12 years old (n = 32)	35.89 ± 3.24	6.10 ± 0.73	1.27 ± 0.15	439.16 ± 10.38	66.80 ± 2.47	46.57 ± 9.90
P-value	0.009**	0.176	0.538	0.576	0.017*	0.904

\* P < 0.05.

\*\* P < 0.01.

\*\*\* P < 0.001.

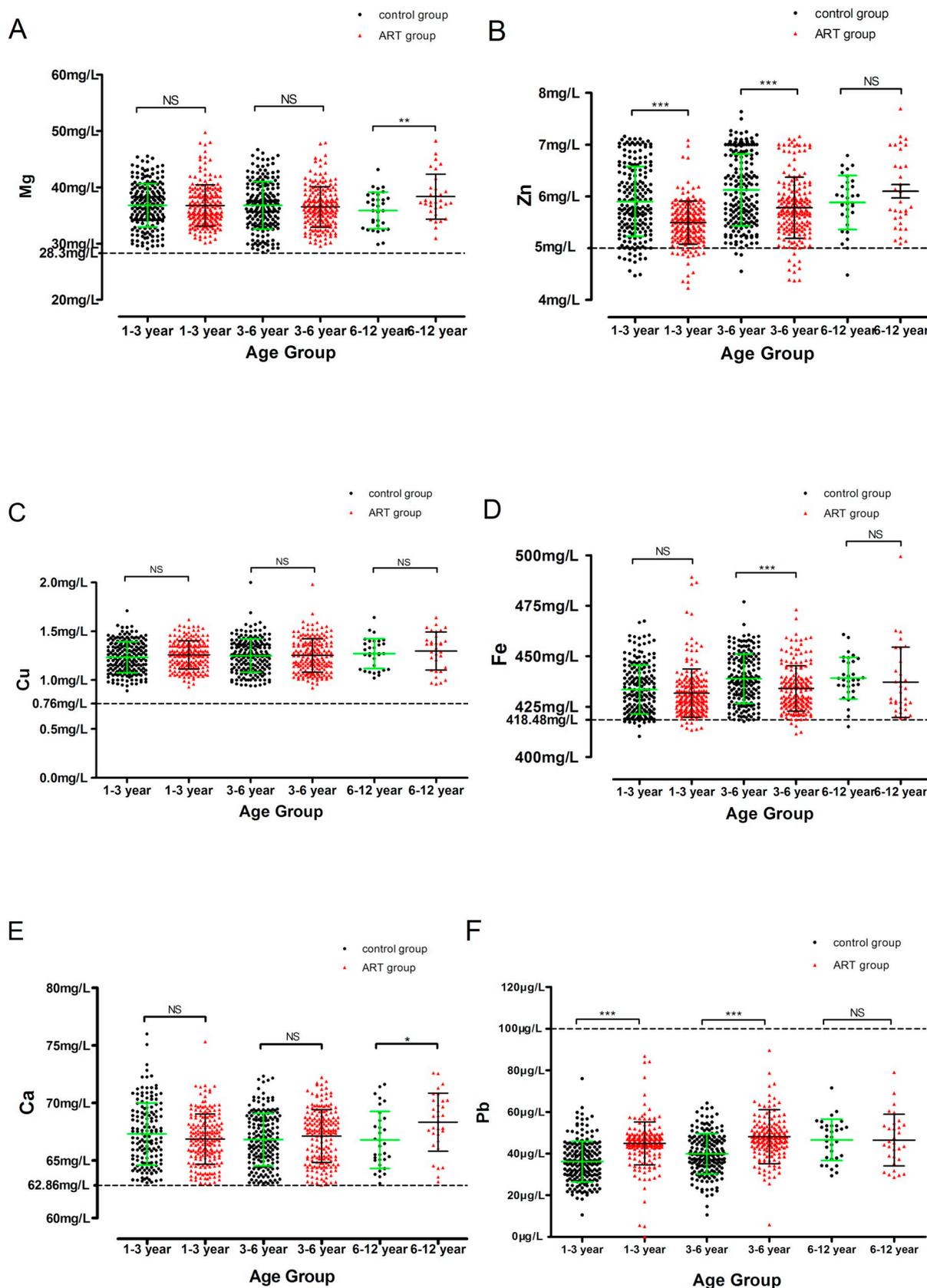
concentration of total body Zn is approximately 2–3 g, with approximately 60% in skeletal muscle and 30% in bone. Zn helps establish indispensable and extensive functions, such as gene expression regulation and cell division, growth and differentiation. Zn also maintains the stability of cell membrane components and molecular structures. Therefore, changes in Zn homeostasis, especially its deficiency, contribute to growth retardation, delayed sexual and skeletal maturation, alopecia, skin lesions, diarrhea, loss of appetite, and increased susceptibility to infection due to immune system defects. Zn has been classified by WHO as a “problem nutrient” which requirements cannot be met without supplementing breast-fed infants with complementary foods after at 6 months of birth [28]. Zn deficiency can interfere with the formation of neural pathways and neurotransmission, thus affecting neural and psychological development (for example, gross and fine motor skills and social skills) and behavior (for example, attention, activity, engagement, and temperament). Zn supplementation provided to infants and children can be used to improve the mental and motor development of infants and children with Zn deficiency [29]. Infancy, pregnancy and lactation are stages during which the Zn requirement is high [28]. Our results showed that Zn content was lower in the 1–3 year-old and 3–6 year-old ART age groups, and 3–6 year-old ART children were shorter than naturally conceived children. This coincidence pointed to a possibility that ART might be related to skeletal development abnormality by decreased Zn concentration. The significance of this difference in Zn concentration between ART and naturally conceived children is further investigation seems variety of compounding factors associated with the cause of infertility could contributed to child development. In this study ART-conceived children were divided into sub-groups based on infertility factors and compared with the control group by one-way analysis of variance (ANOVA) with a Dunnett post hoc test. Multi-factor analysis is required to determine the exact connection between ART and Zn deficiency.

Fe is crucial for oxygen transport, energy production, DNA synthesis, and cellular proliferation. For example, Fe is found in hemoglobin—an oxygen carrier that transports oxygen from the lungs to other tissues and then carries carbon dioxide back to the lungs [30].

Approximately two-thirds of total body Fe is used to synthesize hemoglobin, and the production of red blood cells is affected by iron deficiency. Fe is also a part of myoglobin, a storage protein that stores oxygen for muscle tissue. Iron deficiency is the major cause of anemia [31]. Children and women are at a much higher risk of iron deficiency, which can result in premature birth, poor growth and development of nervous system [32]. A recent study reported that delayed umbilical cord clamping should be performed for all newborn children to increase fetal Fe stores [23]. In 3–6 year-olds, we observed significantly different Fe levels between the oligoasthenoteratozoospermia or azoospermia factor sub-group and the normal group (p = 0.000) and between the oligoasthenoteratozoospermia or azoospermia factor sub-group and the tubal factor sub-group (p = 0.020). Since the average level was still within the normal limits, and there is no report on significant decrease in ART children on hemoglobin, the effect of ART children Fe level should be further study.

Maintaining an adequate Ca intake during childhood and adolescence is necessary for the development of peak bone mass, which is important for reducing the risk of fractures and osteoporosis later in life. The role of Mg is extremely important; the Mg atom in the porphyrin ring is a key component of chlorophyll, and it is a catalyst that participates in nearly all human metabolic processes. In human cells, Mg is the second most important cation after K. Mg has many physiological functions, such as the ability to activate many types of enzymes, inhibit abnormal nerve excitation, and maintain nucleic acid structural stability and membrane potential. It also participates in protein synthesis, muscle contraction and temperature regulation. Mg deficiency leads to decreased protein synthesis, decreased hormone secretion, digestive organ dysfunction, diabetes, hypertension, and nervous system disorders. Although the average levels of Ca and Mg in the ART group of 6–12 year-olds were significantly higher than those in the control group, the sample size was limited, with each group containing only 32 children, and effect of increased Ca and Mg concentration was unclear. Thus the significance of our observation could not be determined.

Pb is a heavy metal toxic to humans and animals, and its long-term



**Fig. 1.** Scatter diagram of the levels of trace elements in the ART and control groups. The levels of Mg (A) and Ca (E) were significantly higher in the ART group at 6–12 years of age. The mean Zn (B) level was lower in the ART group than in the control group at 1–6 years of age. Compared with the paired control groups, the Pb (F) levels were higher in the ART group at 1–6 years of age, and the Fe (D) levels were lower in the ART group at 3–6 years of age. There was no significant difference in the Cu (C) level between the ART and paired control groups. Significant difference by Student's *t*-test: \**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001. NS, no significance. The longer and shorter bars represent the mean and SD (standard deviation). The dotted line parallel to the x-axis indicates the upper or lower limit of the normal range.

survival allows it to persist in the environment [25]. Persistent Pb pollution of air and water is a major environmental problem throughout the course of industrialization in many countries. Gastrointestinal absorption of Pb is higher in children (40–50%) than in adults (3–10%); thus children are more sensitive to Pb exposure [33]. Blood Pb levels can be tested before clinical symptoms appear. The toxic level of Pb in the blood is 100 µg/L or higher, which may result in neurological disorders, cognitive impairment, hypertension and other disorders [25,34]. Potential contacts with heavy metals in cases of endometriosis, spontaneous abortions, preterm births, stillbirths and hypotrophy have also been reported [35]. These findings suggest that Pb may be a contributing factor for infertility. In our study, serum Pb levels were higher in 1–3- and 3–6 year-old children born via ART compared with naturally conceived children. This result may be affected by ART, air quality, diet or water sources. The vast majority of the children we recruited live in Jiangsu Province, thereby ensuring consistency in their environment, which can cause differences in trace elements between two groups of children.

Our results are valuable for understanding the safety of ART in children before puberty. We need to further understand trace element-related diseases in children, especially regarding the early discovery of subclinical symptoms, prevention and treatment, to reduce the incidence of microelement deficiencies or excesses in childhood. However, the mechanisms underlying the increased risks for children conceived by ART are still unclear. Additional longer-term, larger-scaled follow-up studies with multi-factor analysis are required to determine whether ART could be related to iron or zinc deficiency and how these changes affect the health of children.

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#### Conflicts of interest

The authors declare that they have no conflicts of interest related to the contents of this article.

#### Author contributions

XX wrote the first draft of the manuscript. YZ interpreted the data. YH participated in the physical examinations. SJ helped with the science research and writing. HY performed the data analyses. JL and NZ conducted the health tracking survey and acquired the data. JC and LG recruited the participants. YC reviewed and edited the manuscript. JL designed the study. All the authors read and approved the final manuscript.

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