

Exposure to arsenic during pregnancy and newborn mitochondrial DNA copy number: A birth cohort study in Wuhan, China

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1 **Exposure to arsenic during pregnancy and newborn mitochondrial DNA copy**
2 **number: A birth cohort study in Wuhan, China**

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29 **Abbreviations:** Al, aluminium; As, Arsenic; BMI, body mass index; CI, confidence
30 interval; ICC, intraclass correlation coefficient; ICP-MS, inductively coupled plasma
31 mass spectrometry; LOD, limit of detection; Mn, manganese; mtDNAcn,
32 mitochondrial DNA copy number; NO₂, nitrogen dioxide; Pb, lead; qPCR,
33 quantitative real-time polymerase chain reaction; SD, standard deviation; SG, specific
34 gravity; Tl, thallium.

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36

37 **Abstract**

38 **Background:** Arsenic (As) is a widely distributed environmental chemical with
39 potentially different toxicities. However, little is known about the impact of maternal
40 As exposure on newborn mitochondrial DNA copy number (mtDNAcn), which may
41 lie on the pathway linking As exposure to adverse health impacts.

42 **Objectives:** We aimed to explore whether maternal As exposure was associated with
43 newborn mtDNAcn.

44 **Methods:** We conducted a birth cohort study of 762 mother-infant pairs in Wuhan,
45 China, 2013-2015. Cord blood mtDNAcn was determined using qPCR. Maternal
46 urinary As levels in each trimester were quantified by ICP-MS. Multiple informant
47 models were used to examine the associations of repeated urinary As levels with cord
48 blood mtDNAcn.

49 **Results:** The median urinary As levels in the first, second, and third trimesters were
50 17.2 µg/L, 16.0 µg/L and 17.0 µg/L respectively. In the multivariate model, each
51 doubling increase in the first-trimester urinary As level was associated with a 6.6%
52 (95% CI: -12.4%, -0.5%) decrease in cord blood mtDNAcn. The highest versus
53 lowest quintile of first-trimester urinary As level was related to a 19.0% (95% CI:
54 -32.9%, -2.2%) lower cord blood mtDNAcn. There was significant association of
55 urinary As levels in the second and third trimesters with cord blood mtDNAcn. The
56 inverse relationship between first-trimester urinary As level and cord blood mtDNAcn
57 was more pronounced among female infants.

58 **Conclusions:** First-trimester As exposure was associated with decreased cord blood
59 mtDNAcn. The potential health impacts of decreased mtDNAcn in early life need to
60 be further clarified.

61 **Keywords:** Arsenic; maternal exposure; newborns; mitochondrial DNA copy number;

62 trimester-specific

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81 **1. Introduction**

82 Arsenic (As), a widely distributed metalloid element, is a naturally occurring element
83 that exists in both organic and inorganic forms (ATSDR, 2007). The inorganic forms
84 of As are harmful, while most of the organic forms of As are essentially harmless.
85 Human are exposed to As mainly through drinking water and food (WHO, 2018).
86 Upon ingestion, As is metabolized and mainly excreted through urine, and can be
87 measured in urine, blood, or hair (ATSDR, 2007). As levels in urine can reflect
88 ongoing exposures and are well correlated with As intake from food and drinking
89 water (Ahsan et al., 2000; Calderon et al., 1999; Pellizzari and Clayton, 2006). It has
90 been estimated that more than 200 million people worldwide might be chronically
91 exposed to As in drinking water at levels above the World Health Organization (WHO)
92 recommended limit of 10 µg/L (Naujokas et al., 2013). As-contaminated drinking
93 water is widespread and represents a major public health problem worldwide (Kapaj
94 et al., 2006; MM Rahman et al., 2009).

95 Exposure to As is of particular concern among pregnant women and fetuses
96 because they are especially vulnerable to some environmental toxicants (Vahter, 2009).
97 As can easily cross placenta and has been detected in cord blood (Concha et al., 1998;
98 Hall et al., 2007). Extensive evidence suggested that As exposure during pregnancy
99 was associated with adverse pregnancy and birth outcomes (e.g., spontaneous abortion,
100 stillbirth, infant mortality, and fetal growth restriction) (Quansah et al., 2015). These
101 impacts of maternal As exposure on adverse health outcomes have been suggested to
102 result from the increased oxidative stress (Ahmed et al., 2011; A Rahman et al., 2009).

103 Mitochondria, intracellular organelles, are the primary target and major intracellular
104 source of reactive oxygen species (ROS) in animal and human cells (Yakes and Van
105 Houten, 1997). Each animal and human cell consists of several hundreds to a

106 thousand mitochondria, each carrying 2-10 copies of mitochondrial DNA (mtDNA).
107 Compared with nuclear DNA, mtDNA has a high mutation rate and is more
108 susceptible to ROS-induced damage due to lack of protective histone and limited
109 repair capacity (Lee and Wei, 2000; Linnane et al., 1989). It has been reported that
110 mitochondria can compensate for mtDNA oxidative damage by the alteration of
111 mtDNA copy number (mtDNA_{cn}) (Lee et al., 2000; Yakes and Van Houten, 1997),
112 thus mtDNA_{cn} has been considered as a marker of mitochondrial response to damage.
113 MtDNA_{cn} has been inversely associated with aging-related diseases, such chronic
114 kidney disease (Tin et al., 2016), cardiovascular disease (Ashar et al., 2017), and
115 all-cause mortality (Ashar et al., 2015).

116 Low mtDNA_{cn} and mutations in mtDNA resulting from oxidative damage have
117 been reported to persist and accumulate over time (Kujoth et al., 2005; Mengel-From
118 et al., 2014; Sondheimer et al., 2011), indicating that early exposures may influence
119 later mitochondrial health. In addition, decreased mtDNA_{cn} was related to fetal
120 outcomes that are critical predictors of health in later life like intrauterine growth
121 restriction, birth weight, and birth length (Clemente et al., 2016; Clemente et al., 2017;
122 Mando et al., 2014). Thus, identification of the relationships between early-life
123 exposures and newborn mtDNA_{cn} may be a major step forward in unravelling the
124 early-life determinants of diseases in later life. A growing body of studies have been
125 conducted to explore the determinants of newborn mtDNA_{cn}, such as maternal
126 smoking (Bouhours-Nouet et al., 2005), maternal lifetime stress (Brunst et al., 2017),
127 maternal air pollution exposure (particulate matters with aerodynamic diameter ≤ 2.5
128 μm (PM_{2.5}) (Brunst et al., 2018; Rosa et al., 2017) and ≤ 10 μm (PM₁₀) (Janssen et al.,
129 2012), nitrogen dioxide (NO₂) (Clemente et al., 2016; Clemente et al., 2017), and
130 household air pollution (Kaali et al., 2018)), and maternal heavy metal exposure

131 (Kupsco et al., 2019; Liu et al., 2019; Sanchez-Guerra et al., 2019; Vriens et al., 2017;
132 Wu et al., 2019; Xu et al., 2019). To date, only one study from Belgium showed a
133 positive relationship of cord blood As with placental mtDNAcn (Vriens et al., 2017).
134 Evidence regarding the relationships between trimester-specific As exposure and
135 newborn mtDNAcn was lacking.

136 Therefore, we explored whether maternal As exposure during pregnancy was
137 related to cord blood mtDNAcn and identified the sensitive exposure windows in a
138 birth cohort study.

139 **2. Material and methods**

140 *2.1. Study population*

141 This birth cohort study was conducted between November 2013 and March 2015 at
142 the Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital) in
143 Wuhan city, Hubei province, China. Briefly, 762 mother-infant pairs were enrolled if
144 the mothers met the following criteria: 1) residing in Wuhan City, 2) a singleton
145 gestation with <16 weeks of pregnancy at enrollment, and 3) being willing to deliver
146 at study hospital and planning to attend prenatal examination. We excluded 16
147 participants with missing cord blood samples or ineligible DNA quality, 746
148 mother-infant pairs were included for the final analysis. Of the 746 included pregnant
149 women, 598 (80.2%) provided urine samples at all three trimesters. The number of
150 women with urine samples in the first, second, and third trimester were 746, 745, and
151 599, respectively.

152 The study was approved by the ethics committees of Tongji Medical College,
153 Huazhong University of Science and Technology and the Wuhan Children's Hospital
154 (Wuhan Maternal and Child Healthcare Hospital). All participants signed informed
155 consent.

156 2.2. *Urine collection and exposure measurements*

157 Spot urine samples were collected from pregnant women in the first (mean \pm standard
158 deviation (SD), 13.0 ± 1.1 weeks), second (23.6 ± 3.2 weeks), and third trimesters
159 (35.1 ± 3.1 weeks). Urine samples were collected in polypropylene tubes and were
160 frozen at -20 °C until analyses.

161 Maternal As exposures were estimated using urinary As levels. Total urinary As
162 levels (including organic and inorganic As) were measured using inductively coupled
163 plasma mass spectrometry (ICP-MS, Agilent 7700, Agilent Technologies). Urinary
164 levels of lead (Pb), aluminium (Al), manganese (Mn), and thallium (Tl) were also
165 quantified by ICP-MS, because they were reported to be associated with newborn
166 mtDNAcn (Kupsco et al., 2019; Liu et al., 2019; Sanchez-Guerra et al., 2019; Wu et
167 al., 2019). The detailed methods of measurement and quality control have been
168 described previously (Liu et al. 2018). In brief, urine samples were thawed at room
169 temperature and then nitrated overnight by 3% HNO_3 . The resulting samples were
170 digested by ultrasound at 40 °C for 1h. The limits of detection (LOD) for urinary As,
171 Pb, Al, Mn, and Tl were 0.020 $\mu\text{g/L}$, 0.008 $\mu\text{g/L}$, 0.106 $\mu\text{g/L}$, 0.050 $\mu\text{g/L}$, and 0.020
172 $\mu\text{g/L}$, respectively. One urinary arsenic concentration in this study was below the
173 LOD, which was replaced as $\text{LOD}/\sqrt{2}$. The intra-day and inter-day coefficients of
174 variation for urinary As, Pb, Al, Mn, and Tl were 0.286%-0.858% and
175 0.272%-2.584%, respectively.

176 Urinary specific gravity (SG) was measured by a refractometer (Atago PAL-10S;
177 Atago, Tokyo, Japan). Levels of urinary As and other metals (Pb, Al, Mn, and Tl)
178 were corrected to control for variations in urine dilution by SG according to the
179 following formula: $P_{SG} = P[(1.012-1)/(SG-1)]$, where P_{SG} is the SG-corrected
180 exposure levels, P is the measured exposure levels, the value of 1.012 is the median

181 SG in this study population, and SG is the specific gravity of the individual urine
182 samples.

183 2.3. Measurements of mtDNAcn

184 Cord blood was collected immediately at delivery. Blood samples were centrifuged
185 and placed at -80 °C until DNA extraction. DNA was isolated from the leukocytes of
186 umbilical cord blood samples by Wizard[®] Genomic DNA Purification (Promega
187 Corporation, Madison, WI, USA). Relative cord blood mtDNAcn was measured by
188 quantitative real-time PCR (qPCR) assay and the sequences of primer, reaction
189 mixture, and PCR thermal cycling profile are described previously (Liu et al., 2019;
190 Wu et al., 2019). In brief, relative cord blood mtDNAcn was calculated by the ratio of
191 the mitochondrial gene copy numbers (*mtND1*) to the single-copy nuclear control
192 gene [human beta-globin (*hbg*)]. All measurements were conducted in triplicates using
193 the ViiA[™] 7 Dx Real-Time PCR System (Applied Biosystems) in 384-well plates. A
194 pool of 50 genomic DNA samples selected randomly from our study population was
195 used to construct a standard curve with five-point serial dilution, ranging from 104
196 ng/μL to 0.4 ng/μL ($R^2 \geq 0.99$). To ensure quality control, each plate included standard
197 curve, negative controls, and inter-plate controls. The intra-run and inter-run
198 coefficients of variation for mtDNAcn measurements were 2.8% and 3.8%,
199 respectively.

200 2.4. Covariates

201 Information on socio-demographic characteristics and lifestyle behaviors during
202 pregnancy was collected via standard questionnaires, including maternal age,
203 education, occupation, alcohol consumption during pregnancy, and active and passive
204 smoking during pregnancy. Information on parity, infant sex, and birth date was
205 obtained from medical records. Season of birth was categorized into warm period

206 (June-November) and cold period (December-May). Gestational age at birth was
207 determined based on the last menstrual period. Pre-pregnancy body mass index (BMI)
208 was estimated according to the ratio of pre-pregnancy weight (kg) to height squared
209 (m^2).

210 *2.5. Statistical analysis*

211 Continuous data were shown as mean \pm SD (normally distributed) or median with
212 25-75th (not normally distributed), and categorical data as number (frequency).
213 Urinary As levels and mtDNAcn were ln-transformed to improve the normality. We
214 computed Spearman correlation coefficients of uncorrected and SG-corrected urinary
215 As levels in three trimesters. Reproducibility of urinary As levels in three trimesters
216 was estimated using the intraclass correlation coefficient (ICC). The ICC was
217 determined by dividing the between-person variance by the sum of within- and
218 between-person variances. The ICC values of < 0.40 , $0.40-0.75$, and > 0.75 were
219 defined as weak, moderate, and strong reproducibility, respectively (Rosner, 2011).

220 Multiple informant models (Sanchez et al., 2011) were applied to examine the
221 trimester-specific relationships between urinary As levels and cord blood mtDNAcn.
222 The multiple informant models not only simultaneously assessed the association
223 between As exposure in each trimester and cord blood mtDNAcn in the same model,
224 but also tested homogeneity in relationships of maternal As exposure with cord
225 blood mtDNAcn across three trimesters. We assessed the association of maternal As
226 exposure with cord blood mtDNAcn in two ways: continuous (ln-transformed
227 maternal As level) and categorical (quintiles of maternal As level) variables. *P*-values
228 for trend were determined by fitting the median value of each quintile as a continuous
229 variable. Covariates were chosen based on existing studies or they could lead to
230 changes of main effect by $>10\%$ (Greenland, 1989). Multivariate models were

231 adjusted for maternal age (continuous), pre-pregnancy BMI (continuous), education
232 (junior high school or below/high school/college or above), parity (primiparous/
233 multiparous), occupation (employed/unemployed), passive smoking (yes/no), infant
234 sex (male/female), and gestational age (continuous). To improve interpretability of
235 regression analyses consisting of ln-transformed exposure and/or outcome variables,
236 we calculated the percent change and 95% confidence interval (CI) in cord blood
237 mtDNAcn for a doubling or quintile of urinary As.

238 Given that previous studies reported gender differences in the effects of As
239 exposure (Gilbert-Diamond et al., 2016; Kippler et al., 2012), we performed stratified
240 analysis based on infant sex. Tests for interactions were performed using the Wald test
241 (Kaufman and MacLehose, 2013). We also conducted a series of sensitivity analyses:
242 additional adjustment for month of birth, season of birth, and gestational age at urine
243 collection individually; additional adjustment for other metals (Pb, Al, Mn, and Tl);
244 excluding mothers aged ≥ 35 years (advanced maternal age). To explore the possible
245 misclassification induced by the missing urine samples, we also repeated our
246 regression models by restricting to participants with all three urine samples.

247 All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc.,
248 Cary, NC). A two-tailed P -value < 0.05 was considered significant.

249 **3. Results**

250 Characteristics of 746 mother-infants pairs are displayed in Table 1. Mean
251 pre-pregnancy BMI was 20.8 ± 2.8 kg/m² and mean maternal age was 28.6 ± 3.3 years.
252 Among the mothers, 589 (79.0%) had higher education attainment, 446 (59.8%) were
253 employed, 244 (32.7%) were passively exposed to cigarette smoking, and 644 (86.3%)
254 were primiparous. The newborns (383 males and 363 females), including 18 (2.4%)
255 preterm infants, had a mean gestational age at birth of 39.4 ± 1.2 weeks. The

256 characteristics of participants did not differ significantly between mothers with urine
257 samples in all three trimesters and those with missing urine sample, except for
258 education, passive smoking during pregnancy, and season of birth (Table S1).

259 Table 2 presents the distributions, reproducibility (ICCs), and Spearman correlation
260 coefficients of the uncorrected and SG-corrected urinary As levels across three
261 trimesters. The median (25th-75th percentile) levels of SG-corrected urinary As were
262 17.2 (12.4-25.6) $\mu\text{g/L}$ for the first trimester, 16.0 (11.7-24.3) $\mu\text{g/L}$ for the second
263 trimester, and 17.0 (12.2-24.1) $\mu\text{g/L}$ for the third trimester. The SG-corrected ICC of
264 urinary As levels across three trimesters was 0.16, suggesting a week reproducibility.
265 The Spearman's correlation coefficients among urinary As levels during three
266 trimesters ranged from 0.15 to 0.19. The distributions of urinary Pb, Al, Mn, and Tl
267 levels in three trimesters are shown in Table S2. Spearman correlation coefficients of
268 urinary levels of As and other metals in three trimesters are shown in Table S3.

269 Table 3 shows the association of maternal urinary As level with cord blood
270 mtDNAcn. In the unadjusted model, a doubling increase in the first-trimester urinary
271 As level was associated with a 6.6% (95% CI: -12.5%, -0.4%) decrease in cord blood
272 mtDNAcn. After adjustment for potential confounding factors, the association was not
273 materially changed, equivalent to a 6.6% (95% CI: -12.4%, -0.5%) decrease in cord
274 blood mtDNAcn for each doubling increase in the first-trimester urinary As level. The
275 trimester-specific relationships between maternal urinary As levels and cord blood TL
276 were found ($P_{\text{int}} = 0.044$). We also assessed the association between quintiles of
277 maternal urinary As level and cord blood mtDNAcn (Fig. 1 and Table S4). Compared
278 to the lowest quintile, the highest quintile of the first-trimester urinary As level had a
279 19.0% (95% CI: -32.9%, -2.2%) decrease in cord blood mtDNAcn, with a
280 significant dose-response relationship across these quintiles (P for trend = 0.041). No

281 significant associations between urinary As levels in the second and third trimesters
282 and cord blood mtDNAcn were observed.

283 In analysis stratified by infant sex (Table 4), first-trimester urinary As level was
284 inversely related to cord blood mtDNAcn (percent change, -10.4%; 95% CI, -19.0%,
285 -1.0%) among female infants, but not male infants (percent change, -3.2%; 95% CI,
286 -10.7%, 5.1%). In the sensitivity analyses, the inverse association between
287 first-trimester urinary As level and cord blood mtDNAcn was not materially changed
288 with further adjustment for month of birth, season of birth, and gestational age at
289 urine collection individually; additional adjustment for other metals (Pb, Al, Mn, and
290 Tl); excluding mothers aged ≥ 35 years; or restricting the analyses to mothers with all
291 three urine samples (Table S5).

292 **4. Discussion**

293 To our knowledge, this is the first report to explore the effects of trimester-specific As
294 exposure on cord blood mtDNAcn. We found that first-trimester As exposure was
295 related to decreased cord blood mtDNAcn, particularly among female infants. No
296 significant associations between maternal As exposure in the second and third
297 trimesters and cord blood mtDNAcn were observed.

298 Almost all of the pregnant women had detectable urinary As levels, suggesting that
299 our study participants were widely exposed to this metalloid element. Urinary As
300 levels in our study (median, 16.6 $\mu\text{g/L}$) were higher than those reported among
301 pregnant women from the United States (median, 3.4-4.3 $\mu\text{g/L}$) (Farzan et al. 2016;
302 Gilbert-Diamond et al. 2016; Gossai et al. 2015) and Canada (Thomas et al. 2015),
303 and were lower than those reported from the Bangladesh (median, 81-94 $\mu\text{g/L}$)
304 (Rahman et al. 2011; Tofail et al. 2009), Japan (geometric mean, 76.9 $\mu\text{g/g}$ creatinine)
305 (Shirai et al. 2010), Mexico (median, 23.3 $\mu\text{g/L}$) (Laine et al. 2015), and Chile

306 (median, 30.3-61.7 $\mu\text{g/g}$ creatinine) (Hopenhayn et al. 2003). In comparison with
307 general population, our study population had higher As levels than those reported
308 from the United States (median, 7.5 and 8.7 $\mu\text{g/g}$ creatinine for men and women)
309 (Kuo et al. 2015), and had lower As levels than those reported from the Spain (median,
310 52.1 $\mu\text{g/g}$ creatinine) (Navarro Serrano et al. 2016) and Bangladeshi (median, 257
311 $\mu\text{g/g}$ creatinine) (Howe et al. 2016). The difference in urinary As levels in different
312 regions were possibly due to the variations in food intake, lifestyle factors, and
313 environmental contamination. As has a short biological half-life in urine and is rapidly
314 metabolized (ATSDR, 2007). However, as far as we know, no studies have reported
315 the temporal variability in urinary As levels over pregnancy. In our study, we observed
316 that the ICC for urinary As levels was 0.16, suggesting a week reproducibility during
317 pregnancy. The week reproducibility was possibly due to the biochemical or
318 physiological changes related to progression of pregnancy (Abduljalil et al., 2012) or
319 the change in external exposure level of As.

320 Drinking water and diet are two major routes for As exposure among general
321 population (WHO, 2018). Our participants were recruited in Wuhan, an inland city in
322 China. All participant are urban residents of Wuhan city and use the municipal tap
323 water as the source of drinking water with lower arsenic level (1-3 mg/L) (Sun et al.
324 2017), which is below the WHO safety standard of 10 $\mu\text{g/L}$ (WHO, 2011). In addition,
325 the staple food of residents is rice in Wuhan city. Rice consumption has been reported
326 to be related to urinary As levels among pregnant women and general population
327 (Gilbert-Diamond et al., 2011; Islam et al., 2016).

328 Evidence on maternal exposure to environmental pollutants associated with
329 newborn mtDNAcn has begun to accumulate, but the direction of effects from
330 different pollutants varied. Studies reported increased newborn mtDNAcn in

331 association with maternal exposure to lead (Sanchez-Guerra et al., 2019), aluminum
332 (Liu et al., 2019), and manganese (Kupsco et al., 2019). Decreased newborn
333 mtDNAcn was reported in relation to maternal smoking (Bouhours-Nouet et al., 2005),
334 maternal thallium exposure (Vriens et al., 2017; Wu et al., 2019), and maternal air
335 pollution exposure [PM_{2.5} (Brunst et al., 2018; Rosa et al., 2017), PM₁₀ (Janssen et al.,
336 2012), NO₂ (Clemente et al., 2016; Clemente et al., 2017), and household air pollution
337 (Kaali et al., 2018)]. The variation in the association of environmental exposures with
338 newborn mtDNAcn is possibly due to variations in exposure level, type of exposure,
339 exposure duration, and exposure population. However, few studies have examined the
340 association of maternal As exposure with newborn mtDNAcn. Contrary to our
341 findings, a research of 233 mother-infant pairs performed by Vriens et al. showed that
342 cord blood As level was related to increased placental mtDNAcn (Vriens et al., 2017).
343 The difference in As level may be the reason for the inconsistent findings. Urinary As
344 level in Vriens's study was much lower than that in our study (mean, 1.19 µg/L vs
345 22.9 µg/L). Another possible reason was the different timing of As measurements.
346 Urinary As levels in three trimesters were assessed in our study, while Vriens's study
347 assessed As level using cord blood collected after delivery, representing the short-term
348 neonatal exposure.

349 Although the underlying mechanisms by which maternal As exposure can lead to
350 decreased cord blood mtDNAcn are not fully understood, one plausible explanation is
351 the generation of oxidative stress induced by As exposure (Ahmed et al., 2011;
352 Jomova et al., 2011). Compared with nuclear DNA, mtDNA is especially prone to
353 oxidative damage due to lack of protective histone and lower repair capacity (Lee and
354 Wei, 2000; Linnane et al., 1989). Mitochondria can respond to mtDNA oxidative
355 damage by increasing mtDNAcn (Lee and Wei, 2005). However, with increasing

356 mtDNA oxidative damage, the compensatory mechanism may be deficiency, leading
357 to decrease in mtDNAcn (Lee and Wei, 2005). Furthermore, mitochondria are mainly
358 responsible for cellular energy production (ATP levels). MtDNAcn is related to the
359 size and number of mitochondria (Lee and Wei, 2005), which can alter under different
360 energy demands. Experimental studies have reported that exposure to As was related
361 to reduced Ca^{2+} -ATPase activity (Majumdar et al., 2011; Muthumani and Miltonprabu,
362 2015), which led to decrease in energy production. Increases in energy demands can
363 overwhelm the mitochondria and result in decreased mtDNAcn.

364 In the present study, we observed that first-trimester As exposure was associated
365 with decreased cord blood mtDNAcn, suggesting that mtDNAcn may be susceptible
366 to As exposure during the early developmental stages of fetus. Early pregnancy is
367 generally a critical period to environmental exposures, and the developing fetus is
368 susceptible to the oxidative stress. Once the utero-placental circulation is established,
369 the fetus becomes more resistant to oxidative stress by increasing antioxidant defenses
370 (Dennerly, 2007), which can protect the mitochondria.

371 Interestingly, we found that the effect of maternal As exposure in the first trimester
372 on cord blood mtDNAcn was female-specific. Although the potential mechanisms are
373 yet to be elucidated, it has been recently reported that increased AQP9 gene
374 expression may lead to increased As transport in female fetal placenta but not in male,
375 suggesting that AQP9 gene may be upregulated in response to As in a female
376 sex-specific manner (Winterbottom et al., 2017).

377 Our study has several strengths, including large sample size, prospective design,
378 and detailed covariates. In addition, we measured repeated maternal urinary As levels,
379 which helped to clarify the effect of maternal As exposure on newborn mtDNAcn
380 with more precision. Moreover, multiple informant models were applied to examine

381 the relationship of maternal As exposure with newborn mtDNAcn, which enabled us
382 to identify the sensitive windows of maternal As exposure for newborn mtDNAcn.

383 Our study has limitations. First, we did not distinguish different As species or
384 metabolites in urine samples, and therefore our results represent the effects of both
385 inorganic and organic As exposures. Second, although we carefully adjusted for
386 several potential confounders, it is not possible to entirely rule out the possibility of
387 residual confounding by unobserved factors in relation to As exposure and mtDNAcn.
388 Third, all study population in the current study were Chinese, which may limit the
389 generalizability of our findings.

390 **5. Conclusion**

391 Our study provided evidence that maternal As exposure in the first trimester was
392 related to decreased newborn mtDNAcn, which suggests a sensitive window for
393 maternal As exposure. Future investigations are needed to further explore the effects
394 of As-related decrease in mtDNAcn at birth on subsequent health of offspring.

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397 **Conflict of interest**

398 The authors declare they have no actual or potential competing financial interests.

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Table 1. Characteristics of mother-infant pairs (n=746).

Variables	Mean \pm SD, geometric mean (25th-75th percentile) or n (%)
Maternal characteristics	
Maternal age (years)	28.6 \pm 3.3
Pre-pregnancy BMI (kg/m ²)	20.8 \pm 2.8
Education	
Junior high school or below	42 (5.6)
high school	115 (15.4)
College or above	589(79.0)
Occupation	
Employed	446 (59.8)
Unemployed	294 (39.4)
Missing	6 (0.8)
Alcohol use during pregnancy	
Yes	0 (0.0)
No	746 (100.0)
Smoking during pregnancy	
Yes	0 (0.0)
No	746 (100.0)
Passive smoking during pregnancy	
Yes	244 (32.7)
No	502 (67.3)
Parity	
Primiparous	644 (86.3)
Multiparous	102 (13.7)
Infant characteristics	
Infant sex	
Male	383 (51.3)
Female	363 (48.7)
Gestational age (weeks)	39.4 \pm 1.2
Preterm birth (< 37 weeks)	
Yes	18 (2.4)

No	728 (97.6)
Season of birth	
Warm period (June-November)	393 (52.7)
Cold period (June-November)	353 (47.3)
Cord blood mtDNAcn	1.2 (0.7–2.2)

659 Abbreviations: BMI, body mass index; mtDNAcn, mitochondrial DNA copy number.
660 Continuous variables are presented by mean \pm SD (normally distributed) or geometric
661 mean with 25-75th percentile (not normally distributed); categorical variables are
662 expressed by n (%).

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Table 2. Distributions and intraclass correlation coefficients, and spearman correlation coefficients of maternal urinary arsenic levels across three trimesters.

Arsenic levels	GM (95% CI)	Percentile			1st trimester	2nd trimester	3rd trimester	ICC
		25th	50th	75th				
Uncorrected ($\mu\text{g/L}$)								0.16
1st trimester	16.6 (15.5, 17.7)	9.6	17.8	30.0	1.00			
2nd trimester	13.8 (12.9, 14.7)	7.6	13.4	24.9	0.17	1.00		
3rd trimester	13.2 (12.4, 14.1)	7.6	12.7	22.9	0.16	0.15	1.00	
SG-corrected ($\mu\text{g/L}$)								0.16
1st trimester	18.3 (17.5, 19.2)	12.4	17.2	25.6	1.00			
2nd trimester	17.4 (16.6, 18.2)	11.7	16.0	24.3	0.18	1.00		
3rd trimester	18.0 (17.1, 18.9)	12.2	17.0	24.1	0.19	0.16	1.00	

Abbreviations: CI, confidence interval; GM, geometric mean; ICC, intraclass correlation coefficient; SG, specific gravity.

1 Table 3. Associations between maternal arsenic exposure during pregnancy and cord
 2 blood mtDNAcn.

Arsenic concentrations (µg/L)	No. of subjects	Percent change (95% CI)	
		Model 1	Model 2
1st trimester	746	-6.6 (-12.5, -0.4)	-6.6 (-12.4, -0.5)
2nd trimester	745	3.3 (-3.3, 10.4)	2.4 (-4.0, 9.3)
3rd trimester	599	4.2 (-4.7, 13.8)	4.2 (-4.7, 13.9)
P_{int}^a		0.035	0.044

3 Abbreviations: CI, confidence interval.

4 Model 1: unadjusted

5 Model 2: adjusted for maternal age, pre-pregnancy BMI, parity, education, occupation,
 6 passive smoking during pregnancy, infant sex, and gestational age.

7 ^a Score test of homogeneity of regression coefficients across three trimesters.

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24 Table 4. Associations between maternal arsenic exposure during pregnancy and cord
 25 blood mtDNAcn, stratified by infant sex.

Arsenic levels ($\mu\text{g/L}$)	Percent change (95% CI)		$P_{sex-int}^b$
	Model 1	Model 2	
Infant sex			
Male (n=383)			
1st trimester	-3.7 (-11.5, 4.8)	-3.2 (-10.7, 5.1)	0.237
2nd trimester	-0.6 (-10.1, 9.8)	-1.9 (-11.0, 8.2)	0.227
3rd trimester	5.2 (-7.3, 19.5)	3.2 (-9.1, 17.2)	0.814
P_{int}^a	0.510	0.615	
Female (n=363)			
1st trimester	-10.1 (-18.9, -0.5)	-10.4 (-19.0, -1.0)	
2nd trimester	7.3 (-2.0, 17.4)	6.6 (-2.8, 16.8)	
3rd trimester	2.9 (-8.6, 15.9)	5.4 (-6.5, 18.7)	
P_{int}^a	0.010	0.006	

26 Abbreviations: CI, confidence interval.

27 Model 1: unadjusted

28 Model 2: adjusted for maternal age, pre-pregnancy BMI, parity, education, occupation,
 29 passive smoking during pregnancy, and gestational age.

30 ^a Score test of homogeneity of regression coefficients across three trimesters.

31 ^b P -value for the interaction between infant sex and maternal arsenic exposure within
 32 each trimester.