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Associations between blood metals and fecundity among women residing in New York State

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Abstract

Trace exposures to metals may affect female reproductive health. To assess the relation between trace concentrations of blood metals and female fecundity, 99 non-pregnant women discontinuing contraception for the purpose of becoming pregnant were prospectively followed. Participants completed a baseline interview and daily diaries until pregnant, or up to 12 menstrual cycles at risk for pregnancy; home pregnancy test kits were used. For 80 women, whole blood specimens were analyzed for arsenic, cadmium, lead, magnesium, nickel, selenium and zinc using inductively coupled plasma mass spectrometry (ICP-MS). Time to pregnancy was estimated using Cox proportional hazards models for discrete time. Metal concentrations were generally within population reference intervals. Adjusted models suggest a 51.5% increase in the probability for pregnancy per 3.60 µg/L increase in Mg (P=0.062), and a 27.7% decrease per 0.54 µg/L increase in Zn (P=0.114). Findings indicate that Mg and Zn may impact female fecundity, but in varying directions.

Keywords

Arsenic (As); Cadmium (Cd); fecundity; Lead (Pb); Magnesium (Mg); Nickel (Ni); Selenium (Se); Zinc (Zn)

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1. Introduction

Environmental factors such as metals and polychlorinated biphenyls (PCBs) may interfere with reproduction in women [1]. Non-essential metals including arsenic (As), cadmium (Cd) and lead (Pb) are widely distributed in the environment and are recognized reproductive toxicants [2]. Exposure to high concentrations of these non-essential toxic trace metals may diminish fecundity, defined as the biologic capacity for reproduction, which is frequently assessed as time-to-pregnancy (TTP) [3-5]. Other metals, including magnesium (Mg), nickel (Ni), selenium (Se), and zinc (Zn) are essential for human reproduction in trace concentrations [6,7]; however, exposure to excess quantities of these elements may also be hazardous [8].

Several metals including As, Cd, Mg, Pb, and Zn have been detected in human follicular fluid underscoring the proximity of these agents to reproductive organs and tissues [9-12]. Studies indicate associations between clinical pregnancy loss and exposure to high concentrations of As [reviewed by [13]]; however, there appear to be no data regarding fecundity and As exposure. Among women undergoing *in vitro* fertilization (IVF), inverse associations have been reported between Pb and Cd concentrations and pregnancy rates [14-18]. In contrast, additional studies suggest positive associations between Cd concentrations, in follicular fluid or urine, and successful IVF outcomes [12,14,19]. A recent, small pregnancy cohort study reported no relation between Pb and retrospectively reported TTP [20]. While equivocal, much of the available data relies upon retrospectively reported TTP whose validity is reported to be low [21], or incomplete quantification of metal exposures, representing a critical data gap in our understanding of human reproduction.

Another critical data gap is the absence of information focusing on the relation between the essential trace metals (Mg, Ni, Se and Zn) and female fecundity, despite longstanding recognition of the deleterious reproductive effects associated with under-nutrition [22]. Several authors have reported significant associations between clinical pregnancy loss and Se deficiencies [6,23-26] or excess [8], as well as Zn deficiency [reviewed by [27]]. Using a prospective pregnancy cohort design with preconception enrollment of women and longitudinal data collection, we sought to address the above data gaps.

2. Materials and Methods

2.1. Study Cohort

One-hundred thirteen women were recruited from a larger cohort of anglers who participated in a survey about species-specific fish consumption and knowledge of New York State fish consumption advisories in 1991–1992 [28]. Women aged 18–34 years, who reported having not completed childbearing, were re-contacted in 1996–1997 to request participation in a prospective pregnancy study. Two-hundred forty-four eligible women were located, including the 113 women who enrolled in this study. However, 14 women were determined to be pregnant prior to study enrollment and were excluded leaving 99 women in the study cohort. A total of 83 women completed the study of which 80 had sufficient blood volume available for the analysis of metals. Previous analysis demonstrated no difference between excluded women and study participants with regard to reproductive endpoints [29], and we have no reason to suspect differences in blood metals concentrations between these groups.

2.2. Longitudinal Data Collection

Study participation included completion of a standardized in-person interview administered by a research nurse in the participant's home and completion of daily diaries designed to

capture menstruation, sexual intercourse and use of cigarettes and alcoholic and caffeinated beverages. Prior to the study, nurses described and reviewed the 'fertile window' with all women and instructed them in the use of home pregnancy kits. Kits were capable of detecting at least 50 mIU/mL of human chorionic gonadotropin (hCG) with sensitivity and specificity >99%, according to the manufacturer, corresponding to levels expected on the first anticipated day of menstruation following conception. A more in-depth description of this study is provided elsewhere [29]. Upon completion of the interview and instructional session, the nurse obtained a 25 mL non-fasting blood specimen that was processed within hours by the participating toxicology laboratory. The nurses followed universal precautions when collecting blood. All equipment was approved by our laboratory as free from contamination. Participants were censored on the day following their first positive pregnancy test or upon completion of 12 menstrual cycles at risk for pregnancy in the absence of pregnancy. An 'at-risk' cycle denoted at least one act of sexual intercourse in the fertile window, which included the five days before through two days after the day of ovulation as estimated by the Ogino-Knaus method [30,31]. Institutional Review Board approval was granted by the University at Buffalo, State University of New York; women provided informed consent prior to enrollment.

2.3. Toxicologic Analysis

Five frozen one-mL aliquots of whole blood per study participant were analyzed for As, Cd, Mg, Ni, Pb, Se, and Zn concentrations according to previously published standardized operating procedures [32-34]. Our laboratory was unable to assess blood Hg due to insufficient sample availability. Briefly, specimens were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) in a modular class 1000 clean laboratory at the University of Notre Dame (Notre Dame, IN). Specimens were pooled by subject in a pre-cleaned and weighed Teflon-Bomb and recorded accordingly. Specimens were analyzed using external calibration and an internal standardization procedure with monitoring for isotopes: ^{75}As , ^{111}Cd , ^{114}Cd , ^{25}Mg , ^{60}Ni , ^{62}Ni , ^{206}Pb , ^{207}Pb , ^{208}Pb , ^{77}Se , ^{82}Se , ^{63}Zn , and ^{65}Zn . Aliquots of caprine serum spiked with known concentrations of analytes were included with each batch to assess measurement reliability (i.e., quality control (QC) specimens). A procedural blank was included in each run to monitor contamination from reagents and laboratory equipment. A bovine liver standard reference material (National Institute of Standards and Technology (NIST) SRM 1577b) was also analyzed with each run to evaluate the accuracy and precision of the technique. Measured values were within the recommended error for the certified values from the NIST in 10 independent digestions and analyses.

Limits of detection (LODs) for metal analytes were calculated as the concentration equivalent to three times the standard deviations of the measured intensities for calibration blanks. These were expressed as $\mu\text{g/L}$ whole blood; 0.005 for As, 0.003 for Cd, 0.006 for Pb, 0.06 for Mg, 0.003 for Ni, 0.012 for Se and 0.03 for Zn. Mean relative standard deviations across and within batches were less than 10% for most analytes including: 6.6% and 5.9% for As, 2.7% and 2.4% for Cd, 5.3% and 8.3% for Pb, 3.0% and 5.7% for Mg, 12.8% and 14.8% for Ni, 5.1% and 5.9% for Se, and 4.9% and 2.4% for Zn, respectively. Concentrations were reported as $\mu\text{g/L}$ whole blood.

2.4. Statistical Analysis

2.4.1 Descriptive and Bivariate Analyses—Descriptive statistics were utilized to inspect the data and to aid in specifying models. All covariates captured by the daily diaries were standardized to a 28-day cycle to address the inherent variability in menstrual cycle length within and across women, and to account for varying TTPs. Distributions were characterized and metals summarized as geometric means and standard deviations and,

subsequently, log-transformed to meet normality assumptions. For metals with an available population reference interval, proportions of values above and/or below the interval were compared for participants with a positive pregnancy test and participants without a positive pregnancy test. For metals with no available population reference interval, proportions of values above the 90th %tile and below the 10th %tile were assessed for participants with and without positive pregnancy tests. Statistical significance was defined as $P < 0.05$ for a two-tailed test based upon the χ^2 -test for categorical variates, ANOVA for continuous variables including metals and PCBs, or the Kruskal-Wallis test for ordinal variables as appropriate. Correlations among metals and PCBs were assessed using Pearson correlation coefficients. All analyses were conducted using SAS version 9.1.3 (SAS Institute, Inc., Cary, NC).

2.4.2 Multivariable Analysis—A Cox proportional-hazards regression model for discrete-time data [35] was used to estimate the effect of individual metal concentrations on TTP. Models included metals following log transformation and potential confounders selected for inclusion using a model fitting algorithm intended to maximize fit inclusive of age (continuous), parity (0 vs. 1+), groupings of PCB congeners (estrogenic PCBs, anti-estrogenic PCBs and ‘other’ PCBs in $\mu\text{g/L}$ serum), serum lipids (mg/dL serum), frequency of intercourse during the fertile window, and use of cigarettes and alcohol standardized to a 28-day cycle. A previously published comprehensive analysis of PCB data suggested that biologic activity groupings [36] of PCB congeners with estrogenic properties (i.e., IUPAC #s 4_10, 5_8, 15_17, 18, 31, 44, 47, 48, 52, 70, 99, 101, 136, 153 and 188) and anti-estrogenic properties (i.e., IUPAC #s 77_110, 105, 114, 126, 171_156 and 169) were inversely and independently associated with TTP [29]. Effects were expressed in terms of model coefficients and their corresponding 95% confidence intervals (CI) in which a positive value for β indicates a shorter TTP, whereas a negative value for β indicates a longer TTP. To facilitate interpretation of these effect estimates in the context of the range of values in the study sample, model coefficients were ‘back-transformed’ to an arithmetic scale, and expressed as the percent change in the conditional probability for a positive pregnancy test, in any given at risk cycle, corresponding to a single interquartile range (IQR) increase in the value of a predictor. The IQR was defined as the difference between the 75th%tile and the 25th%tile for the sample distribution of a variate.

3. Results

3.1. Characteristics of the Study Sample

The 80 participating women with blood metals data available contributed 387 ‘at risk’ cycles for analysis including 27 women who contribute ≤ 1 cycle, 10 who contribute 2 cycles, 7 contributing 3 cycles, 16 women contributing 4-6 cycles, and 20 women contributing > 6 cycles. Fifty-nine (74%) of these women became pregnant while under observation; 46 (78%) pregnancies resulted in live births while 13 (22%) ended in losses at a median of 7 days gestation. Ten (12.5%) women did not conceive during the study period, and 11 women withdrew without pregnancy after contributing a mean ($\pm$ SD) of 4.7 ($\pm$ 4.4) cycles.

Overall, few differences are observed in either the baseline or daily diary characteristics of the cohort between women with a positive pregnancy test and those without as shown in Table 1. A difference is detected for cigarette smoking ($P = 0.007$) and suggested for parity ($P = 0.060$). First, women with positive pregnancy tests reported significantly more cigarettes smoked per cycle while attempting pregnancy than women not achieving pregnancy (i.e., approximately 187 and 22, respectively). Second, restricted to the 60 study participants who reported a pregnancy prior to the inception of this study, 96% of women with a positive pregnancy test had one or more prior births compared with only 4% of women without a positive pregnancy test.

3.2. Concentrations of Blood Metals

With the exception of Mg, no differences are observed in the geometric means for the metals and pregnancy despite all concentrations having been measured above the limits of detection. In addition, again with the exception of Mg, no statistically significant differences are detected in the distribution of values above or below the reference limits, the 90th %tiles, or the 10th %tiles for metals by pregnancy. Overall geometric mean concentrations for blood As (4.27 µg/L), Cd (1.63 µg/L), Ni (6.90 µg/L) and Zn (4.96 µg/L) are measured on the order of parts per billion, with very similar values between those with a positive pregnancy test and those without ($P>0.684$). Blood Pb (15.47 µg/L), Mg (34.06 µg/L) and Se (224.9 µg/L) are measured at higher concentrations overall, on the order of parts per hundred-million and parts per ten-million, respectively, likely reflecting a greater distribution in the environment, including dietary sources, in comparison to As, Cd, Ni, and Zn in spite of a physiologic requirement for the latter pair. Despite statistical similarity, the point estimates for geometric mean blood concentrations for Pb ($P=0.939$) and Se ($P=0.496$) are lower among women with a positive pregnancy test (15.44 and 223.00 µg/L, respectively) than among women without a positive pregnancy test (15.54 and 230.43 µg/L, respectively), and those for As ($P=0.712$), Cd ($P=0.684$) and Ni ($P=0.844$) are higher in the former group (4.33, 1.67 and 6.94 µg/L, respectively) than in the latter group (4.10, 1.52 and 6.81 µg/L, respectively).

Geometric mean concentrations for blood Mg among women with a positive pregnancy test (34.52 µg/L) are statistically greater ($P=0.031$) than those without (32.79 µg/L). Among women with no positive pregnancy test, five participants (23.8%) had blood Mg concentrations <30.35 µg/L (i.e., below the 10th %tile of the sample distribution), compared with three participants (5.1%) among those women with a positive pregnancy test ($P=0.026$). In contrast, concentrations of Zn appear lower among women with a positive pregnancy test (4.93 µg/L blood) compared to women without (5.05 µg/L blood), although not in a statistically significant fashion ($P=0.442$). Overall, most metals are not correlated with each other with the exception of As and Ni ($r=0.54$, $P<0.0001$), As and Se ($r=0.44$, $P<0.0001$), As and Mg ($r=-0.30$, $P=0.007$), and Se and Ni ($r=0.59$, $P<0.0001$). Moreover, none of the metals are significantly correlated with PCBs (data not shown).

3.3. Multivariable Conditional Fecundability Model

After adjusting for potential confounders the effect on TTP varied by metal with some being positive and others negative (Table 3). All 95% CIs included zero, denoting the absence of statistically significant effects at $P<0.05$ (Table 3). However, the results suggest a 51.51% increase in the conditional probability for a positive pregnancy test, in any given at risk cycle for each 3.60 µg/L increase in blood Mg concentrations, a 15.22% increase for each 3.00 µg/L increase in As, a 13.74% increase for each 1.00 µg/L increase in Cd, and a 27.67% decrease for each 0.54 µg/L increase in Zn. The percent change in the conditional probability for a positive pregnancy test per interquartile range increase in blood concentrations of Ni (−8.60%), Se (+8.18%) and Pb (−1.10%) are less than 10%. The regression coefficients from which percent changes are calculated are accompanied by wide confidence intervals indicative of the imprecision of these estimates, although those for Mg and Zn are of ‘borderline’ statistical significance ($P=0.062$ and $P=0.114$, respectively). Of all the covariates assessed, only the sum of anti-estrogenic PCB concentrations (−39.72% per 0.10 µg/L serum), and frequency of intercourse during the fertile window (+111.70% per 2 acts) are statistically significant predictors ($P=0.035$ and $P=0.005$, respectively), whereas the number of alcoholic beverages consumed (−29.67% per 11 drinks consumed) and the number of cigarettes smoked (−0.30% per cigarette) are of borderline statistical significance ($P=0.054$ and $P=0.059$, respectively) in this model.

4. Discussion

This prospective cohort study with preconception enrollment of women upon discontinuation of contraception for purposes of becoming pregnant does not provide conclusive evidence to support the reproductive toxicity of metals at background concentrations. However, we detect a trend towards an increased probability for pregnancy among women with greater blood Mg concentrations, and a trend towards a decreased probability for pregnancy among women with greater blood Zn concentrations. Opposing patterns of effect are observed for various metals, underscoring the importance of considering a mixture of metals rather than relying on a single exposure in keeping with the nature in which humans are exposed. Metals were selected for inclusion in this study based on previously reported associations with female fertility, or on recognized essential roles in normal human physiology. Our study is strengthened by its prospective design, quantification of exposure prior to conception and the hierarchical data structure including the daily capture of information on potential confounding factors such as cigarette smoking and alcohol consumption reported to adversely affect fecundity [reviewed by [37]].

Of all metals, only Mg and Zn blood concentrations are suggestive of possible significant trends with TTP albeit in opposing directions. A 51.51% increase in the cycle specific conditional probability for pregnancy is suggested for each 3.60 µg/L increase in the covariate adjusted blood Mg concentration, and a 27.67% decrease in the conditional probability of pregnancy is suggested for each 0.54 µg/L increase in the covariate adjusted blood Zn concentration, though with borderline statistical significance (i.e., $P=0.062$ and $P=0.114$, respectively). For blood Mg, this trend is consistent with the unadjusted analysis in which concentrations for women with a positive pregnancy test are 1.05-fold (95% CI 1.01-1.10) that of women with no positive pregnancy test. Moreover, the proportion of women with Mg values below the 10th %tile of the sample distribution is more than four-fold higher among women who did not conceive than among women who did ($P=0.026$). The unadjusted analysis for Zn, in contrast, is not consistent with the multivariable results and indicates no association, suggesting the importance of the multivariable adjustment for additional metals and covariates.

The associations suggested by this study are difficult to interpret, given the dearth of prior investigation into the effects of Mg and Zn on human female fecundity. The results of two previously published preliminary studies are consistent with the suggested positive association between blood Mg concentrations and human pregnancy. In a study of 150 zygotes contributed by 25 IVF patients, investigators reported a statistically significant increase in embryonic cell cleavage (4.2 cells vs. 4.9 cells; $P=0.006$) following a doubling of the Mg^{2+} concentration in growth media [38]. However, the results of this study may be artificially over-powered in that the authors erroneously assumed the zygotes to be independent outcomes despite multiple zygotes per woman in the analysis (i.e., embryonic cell cleavage scores are correlated within woman). Additional but somewhat more tangential evidence is provided by a small case-series of 12 women with a history of infertility or spontaneous pregnancy loss, all of whom conceived following four to six months of dietary Mg and Se supplementation leading to normalization of otherwise abnormal red blood cell Mg and glutathione-S-peroxidase concentrations [39]. Changes in Mg concentrations as a function of sex-steroid hormones are well-recognized; inverse associations have been reported for serum progesterone and testosterone concentrations [40,41]. The latter may be of consequence to our suggested results, though the temporality of the association is reversed and not likely to account for the findings. We identified no prior reports describing the results of studies considering Zn and female fecundity, in spite of the well-recognized association between Zn deficiency, pregnancy complications and adverse birth outcomes [reviewed by [42,43]], and a positive association with male fecundity [reviewed by [44]].

While the exact mechanisms by which metals such as Mg and Zn might exert an effect on female fecundity are unknown, these trace elements are essential for the proper function of metalloenzymes which regulate cell division [45-47] and apoptosis [reviewed by [48]]. In addition, Mg binds to and has a stabilizing effect on cell membranes, proteins and nucleic acids [49], and protects lipoproteins from reactive oxygen species [50] which might compromise plasma membrane integrity [51]. It is tempting to speculate that lower body burdens of Mg lead to a decrease in the activity of enzymes necessary for cell replication, concurrent to increased concentrations of reactive oxygen species which damage cell membranes and compromise a developing conceptus. It is possible that one or both of these mechanisms might underlie the association observed between Mg and fecundity. While speculative, inappropriate Zn concentrations might alter patterns of programmed cell death and, thereby, disrupt embryonic growth and implantation, leading to diminished fecundity.

In addition to the associations suggested for Mg, Zn and fecundity, our results corroborate previously reported associations between PCB exposure, lifestyle factors and TTP in this study population [29,]. Furthermore, our study results underscore the importance of modeling environmental exposures in the context of lifestyle factors that adversely impact human reproduction, particularly cigarette smoking and alcohol consumption as recently reviewed [37]. Moreover, the frequency of intercourse during the fertile window demonstrates a positive and statistically significant association with fecundity, as anticipated, underscoring the validity of our multivariable model. However, no association is indicated between age and fecundity during multivariable modeling, in spite of the well-recognized adverse impact of aging on the probability of pregnancy [52]. We suspect this null result reflects the limited age range of participants who were 25 years of age at least and 35 years of age at most at the start of this study.

Although the toxic nature of As, Cd, Pb, and Ni are well characterized, we detect no suggested association with the probability for a positive pregnancy test in either the bivariate or in the multivariable context. Several prior reports, in contrast, described increased rates of spontaneous pregnancy loss (i.e., 1.5-2.5 fold) in association with chronic exposure to groundwater contaminated by inorganic As [53-55], however, with one exception [56], these studies were conducted in As endemic regions characterized by substantial geogenic groundwater contamination by inorganic species. In contrast, participants in the current study did not reside in a recognized As endemic region and thus measured concentrations are likely to reflect the fairly innocuous organic species [57], although given the unspicated nature of our As analysis we are unable to verify this presumption.

Two recent studies report a decreased probability for pregnancy in association with increasing blood Pb concentrations. In a case-control study of 64 infertile women and 83 controls with proven fertility, an increased adjusted odds for blood Pb concentration >25 µg/L was reported (OR 2.94, 95%CI 1.18-7.34) [15]. However, the mean blood Pb concentration for the control group in that study (31.2 µg/L), was approximately double that for participants in the current study. In a prospective cohort study of couples undergoing IVF, Al Saleh et al. [14] reported an inverse association for blood Pb and oocyte fertilization (OR 0.38, 95%CI 0.14-0.99), although this association did not extend to pregnancy as assessed by hCG testing (OR 0.55 95% CI 0.23-1.31). Again, these associations were reported for concentrations of blood Pb approximately twice those of participants in the current study (medians 30.1 µg/L vs. 15.0 µg/L, respectively). Moreover, the results of this study did not accommodate the correlated nature of IVF endpoints and may thus be spuriously over-powered. In contrast, no association was reported between unadjusted blood Pb concentration and TTP in a retrospective cohort study of 41 primigravid women with blood Pb concentrations similar to those measured in our study (median 12.0 µg/L) [20]. An earlier study of 121 women with occupational exposure [17] reported no association

between covariate adjusted blood Pb concentrations ranging from to <103.6-497.3 µg/L and retrospectively reported time to pregnancy (relative risk 0.82, 95% CI 0.52-1.31). We are unaware of any prior studies evaluating the effects of Ni on human female fecundity and are, consequently, unable to interpret our null result in the context of the literature.

Previous investigators have indicated somewhat paradoxical indications of positive associations between adjusted Cd concentrations measured in follicular fluid [12,14], and even urine [19], and oocyte fertilization among women undergoing IVF; however, no such indication is provided by blood Cd concentrations in the current study, although the effect estimate is indeed positive. In the study by Al-Saleh et al. [14] there was no association suggested for blood Cd concentrations and oocyte fertilization *in vitro* (OR 0.45 95%CI 0.07-2.70), or pregnancy (OR 1.72 95%CI 0.74-4.04) at concentrations approximately 2.5-fold lower than those in the current study (mean 0.62 µg/L). We are unaware of any additional studies which assessed blood Cd in association with female fecundity. We detected two outlying Cd values, though we were unable to determine why. These values, equal to 22 µg/L and 478 µg/L blood, are 21 or 477 interquartile ranges above the median value for the Cd distribution, respectively. To assess the influence of these data points we repeated our statistical analysis following re-assignment of these values to the median for our sample distribution equal to 1.0 µg/L blood with no notable changes in results.

Our inability to observe an adverse effect from known toxic metals may stem from the low and relatively comparable metal concentrations across women irrespective of fecundity. With few exceptions, concentrations of metals measured in this study are within the population reference intervals reported for whole blood [58]. All As (<23.0 µg/L), Ni (1.0-28.0 µg/L) and Pb (<250 µg/L) concentrations in our cohort are within population reference intervals for whole blood, although concentrations of Cd exceed the population reference interval (3.9 µg/L) for eleven (13.8%) women, by 1.1 to 474.1 µg/L, and concentrations of Se exceed the population reference interval (>234.0 µg/L) for 36 (45.0%) women, by 6.0-96.0 µg/L. To our knowledge, there are no population reference intervals available for Mg and Zn measured in whole blood. The majority of Mg [45] and Zn [59] ions are sequestered within cells, with concentrations being tightly regulated by a variety of passive and active transport mechanisms. There is a substantial degree of inter-compartment variation in Mg and Zn concentrations, which precludes simple reference interval extrapolation from one matrix (i.e., plasma) to another (i.e., whole blood). Recent estimates do, however, suggest that a large proportion of American women are in fact Mg deficient, a consequence of modern food processing, preservation and packaging technologies [45].

The results of the current study are limited by several factors, foremost of which is the limited sample size available for analysis. We conducted *post hoc* power analyses to determine cohort size of sufficient statistical power to assess Mg and Zn relative to pregnancy at background concentrations for future research initiatives and to help interpret our results. To this end, we estimated that a cohort size comprising 91 and 155 women are sufficient for assessing the Mg and Zn related effects, respectively [60,61]. Given our limited sample, we were unable to assess outcomes such as pregnancy loss that are woman level and not cycle level events. An additional and important limitation is our consideration only of female exposure and covariate data in this study. Ignoring male factors may have introduced exposure misclassification, as recent evidence indicates that male metals exposure may be even more relevant to conception than female exposure [19], as well as unmeasured confounding. Our exposure assessment comprises the simultaneous consideration of several metals, an approach which is more likely consistent with the nature by which human exposure occurs than is represented by studies considering exposure to metals in isolation. Our approach of specifying a unique regression variable for each metal in the absence of observed collinearity allowed us to simultaneously assess these exposures

in relation to TTP. However, our use of single preconception measure of blood metals concentrations may have introduced exposure misclassification for non-persistent metals, those other than Cd or Pb. Whereas the results of this study are only suggestive and useful for hypothesis generation, our detection of potential effects for Mg and Zn on human fecundity is of importance.

The results of this prospective cohort study with preconception enrollment of women and longitudinal capture of covariates relevant for TTP are suggestive of a possible relation with Mg and Zn, although in opposing directions with Mg shortening and Zn lengthening TTP. These results represent the first report of associations between Mg, Zn and human female fecundity in the scientific literature, to the best of our knowledge. The imprecision of regression coefficients as reflected by the wide confidence intervals underscores the importance of cautious interpretation. Still, we believe these results merit a more rigorous consideration of the potential impact of Mg and Zn exposures on female fecundity using a larger sample size with sufficient statistical power.

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Table 1

Comparison of cohort by result of pregnancy testing (n=80).

Characteristic	No positive pregnancy test, n (%)	Positive pregnancy test, n (%)
Age (years):		
25-29	8 (38.1)	22 (37.3)
30-35	13 (61.9)	37 (62.7)
Mean ($\pm$ SD)	30.29 ($\pm$ 2.74)	30.29 ($\pm$ 2.33)
Education:		
High School	3 (14.3)	6 (10.2)
College	16 (76.2)	43 (72.9)
Graduate school	2 (9.5)	10 (16.7)
Gravidity:		
Nulligravid	7 (33.3)	13 (22.0)
Gravid	14 (66.7)	46 (78.0)
Parity: *		
Nulliparous	10 (47.6)	15 (25.4)
Multiparous	11 (52.4)	44 (74.6)
Mean ($\pm$ SD) BMI (kg/m ²)	25.41 ($\pm$ 5.99)	24.10 ($\pm$ 4.82)
Mean ($\pm$ SD) Frequency of sexual intercourse during the fertile window per cycle ^{a, b}	2.05 (1.24)	1.92 (1.51)
Mean ($\pm$ SD) # cigarettes per cycle ^{a, c, **}	21.90 ($\pm$ 43.21)	186.73 ($\pm$ 179.32)
Mean ($\pm$ SD) # of alcoholic beverages per cycle ^{a, d}	15.45 ($\pm$ 14.59)	9.12 ($\pm$ 10.86)

NOTE: No positive pregnancy test includes 11 women who withdrew from the study without a prospectively observed pregnancy and 10 women without a prospectively observed pregnancy during 12 at risk menstrual cycles; except where noted, none of the above differences achieved statistical significance.

^a Standardized to a 28 day menstrual cycle;

^b n=6 missing values (2 with no positive pregnancy test, 4 with a positive pregnancy test);

^c Among n=25 women who reported smoking while trying to conceive;

^d Among n=69 women who reported consuming alcoholic beverages while trying to conceive;

* P<0.10;

** P<0.05; BMI, body mass index; SD, standard deviation.

Table 2Blood metal concentrations ($\mu\text{g/L}$) by result of pregnancy testing (n=80).

Metal ^a	No positive pregnancy test (n=21)	Positive pregnancy test (n=59)
Arsenic		
Mean ($\pm$ SD)	4.10 ($\pm$ 1.62)	4.33 ($\pm$ 1.84)
Min.-Max.	2.00-9.00	1.00-17.00
Cadmium		
Mean ($\pm$ SD)	1.52 ($\pm$ 1.95)	1.67 ($\pm$ 2.82)
Min.-Max.	0.62-5.00	0.62-478.00
Lead		
Mean ($\pm$ SD)	15.54 ($\pm$ 1.57)	15.44 ($\pm$ 1.23)
Min.-Max.	6.00-34.00	8.00-30.00
Magnesium *		
Mean ($\pm$ SD)	32.79 ($\pm$ 1.14)	34.52 ($\pm$ 1.08)
Min.-Max.	25.60-40.80	29.20-42.10
Nickel		
Mean ($\pm$ SD)	6.81 ($\pm$ 1.44)	6.94 ($\pm$ 1.47)
Min.-Max.	4.09-14.00	4.00-16.00
Selenium		
Mean ($\pm$ SD)	230.43 ($\pm$ 1.21)	223.00 ($\pm$ 1.21)
Min.-Max.	140.00-300.00	150.00-330.00
Zinc		
Mean ($\pm$ SD)	5.05 ($\pm$ 1.11)	4.93 ($\pm$ 1.13)
Min.-Max.	4.17-5.91	3.51-6.49

NOTE: No positive pregnancy test includes 11 women who withdrew from the study without a prospectively observed pregnancy and 10 women without a prospectively observed pregnancy during 12 at risk menstrual cycles;

^a Geometric means and standard deviations;

* $P < 0.05$; Max, highest measured concentration; Min, lowest measured concentration; SD, standard deviation.

Table 3

Regression coefficients and 95% confidence intervals (CI) and percent change in the probability for a positive pregnancy test in any given at risk cycle (n=80) ^a.

Predictor	β ^b	95% CI β		P-value	IQR ^c	% change ^d
		Low	High			
Arsenic	0.253	-0.613	1.119	0.566	3.00	+15.22
Cadmium	0.317	-0.191	0.825	0.221	1.00	+13.74
Lead	-0.031	-1.066	1.004	0.954	6.00	-1.10
Magnesium	4.197	-0.216	8.610	0.062	3.60	+51.51
Nickel	-0.176	-1.475	1.123	0.790	5.00	-8.60
Selenium	0.300	-1.778	2.378	0.777	70.00	+8.18
Zinc	-2.234	-5.004	0.536	0.114	0.54	-27.67
Estrogenic-PCBs	-0.948	-3.548	1.652	0.475	0.41	-35.34
Antiestrogenic-PCBs	-5.886	-11.347	-0.425	0.035	0.10	-39.72
Other-PCBs	0.372	-0.102	0.846	0.124	2.78	+188.48
Lipids	-0.001	-0.004	0.002	0.596	133.51	-0.02
Age	0.066	-0.084	0.216	0.384	3.00	+25.99
Parity	0.574	-0.233	1.381	0.164	1.00	+77.54
Frequency of intercourse	0.250	0.077	0.423	0.005	2.00	+111.70
Alcohol	-0.032	-0.064	0.000	0.054	11.00	-29.67
Cigarettes	-0.003	-0.005	0.000	0.059	1.00	-0.30

^aConditional fecundability analog to the Cox proportional hazards model adjusted for baseline concentrations of metals, PCBs and total serum lipids, and age and parity as fixed covariates, and frequency of intercourse during the fertile window, alcohol use and cigarette use as time-varying covariates;

^bPositive β suggests shorter time to pregnancy, negative β suggests longer time to pregnancy;

^cInterquartile range (IQR) is equal to the difference between the value at the 75thtile and the 25thtile of the sample distribution for a variate;

^dIndicates the percent change in the probability for a positive pregnancy test for an increase in value equal to one IQR.