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Low-level arsenic exposure via drinking water consumption and female fecundity - a preliminary investigation

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Abstract

High level arsenic exposure is associated with reproductive toxicity in experimental and observational studies; however, few data exist to assess risks at low levels. Even less data are available to evaluate the impact of low level arsenic exposure on human fecundity. Our aim in this pilot study was a preliminary evaluation of associations between low level drinking water arsenic contamination and female fecundity. This retrospective study was conducted among women previously recruited to a hospital-based case-control study of spontaneous pregnancy loss in Timiş County, Romania. Women (n = 94) with planned pregnancies of 5–20 weeks gestation completed a comprehensive physician-administered study questionnaire and reported the number of menstrual cycles attempting to conceive as the time to pregnancy (TTP). Drinking water samples were

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collected from residential drinking water sources and we determined arsenic levels using hydride generation-atomic absorption spectrometry (HG-AAS). Multivariable Cox-proportional hazards regression with Efron approximation was employed to evaluate TTP as a function of drinking water arsenic concentrations among planned pregnancies, adjusted for covariates. There was no main effect for drinking water arsenic exposure, yet the conditional probability for pregnancy was modestly lower among arsenic exposed women with longer TTPs, relative to women with shorter TTPs, and relative to unexposed women For example, 1 μg/L average drinking water arsenic conferred 5%, 8%, and 10% lower likelihoods for pregnancy in the 6th, 9th, and 12th cycles, respectively (P = 0.01). While preliminary, our results suggest that low level arsenic contamination in residential drinking water sources may further impair fecundity among women with longer waiting times; however, this hypothesis requires confirmation by a future, more definitive study.

Keywords

arsenic; fecundity; pregnancy; Romania; reproduction; water

1. Introduction

Chronic arsenic exposure is linked to myriad human health outcomes, affecting virtually every biologic system, including reproduction (ATSDR, 2007; Naujokas et al., 2013). Millions worldwide are exposed to arsenic contaminated drinking water exceeding 10 μg/L, the maximum contaminant level (MCL) set by the World Health Organization (WHO) (Smedley and Kinniburgh, 2002; WHO, 2006). Arsenic levels exceeding 10 μg/L or even 50 μg/L and higher, have been found in groundwater aquifers in Argentina, Bangladesh, Chile, China, Hungary, India, Mexico, and the U.S. among other locations (Smedley and Kinniburgh, 2002; Welch et al., 2000). At these levels of exposure, drinking water arsenic contamination has been linked to spontaneous abortion, stillbirth, lower birth weight, and smaller birth size (Bloom et al., 2010; Bloom et al., 2014b; Quansah et al., 2015). However, many reproductive-aged women worldwide are also likely to be exposed to drinking water arsenic contamination below 10 μg/L (van Halem et al., 2009; Welch et al., 2000), low levels according to the U.S. National Research Council (NRC, 2001). Given its impact on pregnancy outcomes at high doses and a growing concern for adverse non-cancer health effects at low doses (Carlin et al., 2016), drinking water arsenic contamination less than 10 μg/L may also impact women's fecundity; however, virtually no data exist to characterize the effect.

To begin to address this data gap, we conducted a pilot study of low level arsenic exposure through residential drinking water consumption and female fecundity, defined as the biologic capacity to reproduce, and measured by waiting time until a recognized pregnancy was conceived (Larsen, 2005). Our study population comprised women residing in Timiş County, Romania, an area that falls within the Great Hungarian Plain, which is recognized for groundwater sources naturally contaminated by arsenic, and which are frequently used for private and municipal supplies (Neamtiu et al., 2015; Rowland et al., 2011). These preliminary data will be used to further our knowledge and to guide the design of a future,

more comprehensive study to characterize the reproductive toxicity of low level drinking water arsenic contamination in a more definitive fashion.

2. Materials and Methods

2.1 Study population

Our study population comprised pregnant women, of 5–20 weeks completed gestation, originally recruited to a case-control investigation of spontaneous pregnancy loss in Timiş County, Romania (Bloom et al., 2014a). Prenatal care is compulsory and provided to women at no cost in Romania. This includes registering the pregnancy in early gestation (typically within one month of recognition) and follow-up evaluations conducted during the 2nd and 3rd trimesters. We estimate compliance rates at approximately 85%. We enrolled clinically-confirmed spontaneous abortions (n = 150), of not more than 20 weeks completed gestation, as cases, between December 2011 and January 2013. Women with ongoing pregnancies of similar duration to each case (within 1 week) were recruited as control participants (n = 150). All women received care at the Obstetrics and Gynecology Department of the County Emergency Hospital (Bega Hospital), in the city of Timişoara, obstetrical provider to the vast majority of Timiş County residents. Once enrolled, participants completed a physician administered questionnaire to query demographic, socioeconomic, and lifestyle factors, medical, gynecologic, and occupational histories, as well as residential and drinking water consumption histories. We excluded n = 2 planned pregnancies with missing ‘time to pregnancy’ data (TTP), n = 8 pregnancies conceived using fertility treatments, and n = 3 ‘duplicate’ pregnancies (i.e., women from the parent case-control study, who had first participated as a control and later participated as a case). Women reported planned (n = 94) or unplanned (n = 193) pregnancies, and pregnancy planners reported the number of menstrual cycles of intercourse without contraception that it took to conceive. The self-reported number of cycles trying to conceive, or TTP, is a validated and frequently used measure of couple-based fecundity (Joffe, 1997). Clinical data was abstracted from a medical intake form and we collected blood and urine specimens for use in a future analysis. All participants provided informed consent prior to enrolling in the study and the study protocol was approved by the Institutional Review Boards of the County Emergency Hospital in Timişoara and the University at Albany, State University of New York.

2.2 Exposure assessment

Our trained study staff collected drinking water samples from up to two residential sources reported by study participants, as previously described in detail (Bloom et al., 2014a). Briefly, we collected a 50 mL water sample into screw-top containers previously demineralized with nitric acid (HNO_3) and then rinsed. Concentrated analytical grade HNO_3 (100 μL) was added as a preservative immediately following collection. The water samples were stored on ice and delivered to the Environmental Health Center (Cluj-Napoca, Romania) within 72 hours, for analysis. In the lab, 25 mL of sample water was mixed with 10 mL HCl, and 2 mL of aqueous 5% KI and 5% ascorbic acid (m/m). The sample was heated gently to 50 ºC to reduce As (V) to As (III), and then cooled at room temperature after 15 minutes. Following transfer to a 50 mL volumetric flask, the samples were diluted with de-ionized water to volume. $NaBH_4$ (0.3%) dissolved in NaOH (0.1%) and HCL (3%)

(m/v) was added to the sample, which was transported to a quartz cell, heated to 960 ºC to generate hydrides, and analyzed using a Zeenit 700p atomic absorption spectrometer (Analytikjena, Jena, Germany). All laboratory reagents were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (Steinheim, Germany), and Chem Lab (Zedelgem, Belgium). The method detection limit (MDL) was 0.5 μg/L. Calibration standards and quality control procedures were previously described in detail (Bloom et al., 2014a). We censored negative values to zero for characterizing the exposure distributions and reporting summary measures; however, we did not impute values below the MDL during regression analysis, but rather, we used instrument-reported values to avoid introducing bias (Richardson and Ciampi, 2003; Schisterman et al., 2006).

We operationalized three arsenic exposure variables, to maximize sensitivity for detecting modest associations. Average arsenic level (μg/L) was defined as the arithmetic mean level measured in up to two residential drinking water sources. Peak arsenic level (μg/L) was defined as the highest arsenic level measured in up to two residential drinking water sources. Daily arsenic dose (μg/day) was calculated as the product of average arsenic level multiplied by total average reported daily residential drinking water consumption from non-bottled sources during pregnancy, including teas, coffees, other water-mixed beverages, and soups. For women who resided at the study address for the entire duration of follow-up (i.e., time trying to conceive prior to the pregnancy), we calculated “duration of exposure” by subtracting the week of gestation at study enrollment from the reported TTP in month equivalents (i.e., 1 cycle = 1 month), to target pre-conception exposure. For n = 4 women with incomplete follow-up (e.g., moving to the study residence in the midst of trying to conceive prior to the pregnancy), we left censored “duration of exposure” by subtracting the week of gestation at study enrollment from the date relocating to the study address.

2.3 Statistical analysis

2.3.1 Univariate and bivariate analysis—We examined distributions and assessed potential outliers for arsenic exposure variables and covariates. We used Chi-square and Wilcoxon signed rank tests to compare the distribution of demographic factors and reproductive histories, as well as residential drinking water arsenic exposure for planned pregnancies to unplanned pregnancies. TTP was defined as the number of self-reported menstrual cycles trying to conceive prior to a recognized pregnancy. For planned pregnancies, we used Kaplan-Meier survival curves with log-rank test to evaluate unadjusted associations with TTP, individually for each arsenic exposure variable, and for all covariates. We graphed the log of the cumulative hazard function by log TTP [(log (−log) S(t)) vs. (log TTP)] to visually assess the tenability of proportional hazards assumptions for arsenic exposure variables and confounding covariates (Collett, 1994).

2.3.2 Multivariable analysis—We used Cox proportional hazards regression models to evaluate associations between arsenic exposure variables and TTP among pregnancy planners (n = 94), adjusted for confounding covariates (Collett, 1994). The discrete-time analog to the Cox proportional hazards model generated unstable effect estimates, and so we implemented an Efron approximation for tied event times with traditional Cox-proportional hazards modeling. The Efron approximation more accurately estimates regression

coefficients than other methods when ties are frequent but the sample size is limited (Hertz-Picciotto and Rockhill, 1997). In a first step, we used regression models, incorporating cross-product terms between log TTP and arsenic exposure variables or covariates, to further assess violations of the proportional hazards assumption. In a second step, we simultaneously included arsenic exposure, confounding covariates, and all cross-product terms having $P < 0.10$ in the first step, using a backwards stepwise elimination procedure, and retaining in final models only cross-product terms maintaining $P < 0.10$. We constructed separate models using average arsenic level, peak arsenic level, and daily arsenic dose as the predictor of interest. Exponentiation of regression coefficients provides fecundability hazards ratios (FHRs), which indicate a higher conditional likelihood for pregnancy when > 1.0 and a lower conditional likelihood for pregnancy when < 1.0.

We used directed acyclic graphs (DAGs) to select self-reported covariates for inclusion in regression models as confounders (Greenland et al., 1999). We identified relevant variables for inclusion in DAGs as those predictive of arsenic exposure and female fecundity in the literature, including: maternal age in years (ACOG and ASRM, 2014; Howe et al., 1985); cigarette smoking, as having smoked at least 100 lifetime cigarettes (Howe et al., 1985; Hughes and Brennan, 1996; Jain, 2015; Leffondre et al., 2002); body mass index (BMI), as kg/m^2 (Bolúmar et al., 2000; Grodstein et al., 1994; Yilmaz et al., 2009); urban residence, as a proxy for air pollution exposure (Nieuwenhuijsen et al., 2014; Popescu et al., 2011); education, as a proxy for socioeconomic status (no high school diploma (reference) vs. high school diploma/some college/technical school vs. university degree) (Huber et al., 2010); currently married or living as married (Huber et al., 2010); prior pregnancies and prior pregnancy losses (Axmon et al., 2006); and a history of pelvic inflammatory disease (PID) (Westrom et al., 1992). Based on the DAG we included maternal age, cigarette smoking, and education as confounders in multivariable models.

We used SAS v.9.3 (SAS Institute Inc., Cary NC) for the analysis. Statistical significance was defined as $P < 0.05$ for main effects and $P < 0.10$ for interactions, using two-tailed tests.

2.3.3 Sensitivity analyses—In a first sensitivity analysis we included women with ‘unplanned’ pregnancies (n = 193) as having conceived in the ‘zero cycle’ (i.e., TTP = 0), which addresses left truncation associated with unplanned pregnancies (Joffe et al., 2005). In a second sensitivity analysis, we excluded clinically infertile women (n = 13), those reporting TTP > 12 months (ASRM, 2013), as clinically infertile women may comprise a distinct population.

3. Results

Table 1 compares sociodemographic, clinical, and lifestyle factors between planned (n = 94) and unplanned pregnancies (n = 193) included in the current analysis. Among planners, n = 29 (30.9%) conceived in the 1st cycle, half conceived by the 3rd cycle, and the maximum TTP was 72 cycles. Planned and unplanned pregnancies were mostly similar, although the former were more likely to have married ($P = 0.02$) and to have completed university ($P = 0.001$). Planners also had a slightly higher BMI than non-planners (median = 22.21 vs. 21.20 kg/m^2), although not statistically significant ($P = 0.07$). Table 1 also shows that arsenic

exposure was similarly distributed for planned and unplanned pregnancies. Pregnancy planners were exposed to an average of 4.63 μg/L arsenic in residential drinking water sources (4.11 μg/L for non-planners), with a range of 0 to 175.10 μg/L; however, 90% of women were exposed to ≤ 8.74 μg/L on average. Still, n = 2 were exposed to water sources with an average arsenic concentration > 50 μg/L.

We conducted bivariate analysis for all potential confounding variables (i.e., those included in multivariate models based on DAGs and those not included in multvariate models) using Kaplan-Meier survival curves to evaluate unadjusted associations between TTP, arsenic exposure variables, and covariates, including age, education, cigarette smoking, BMI, urban vs. rural residence, marital status, prior pregnancies, prior pregnancy losses, and a history of PID (Figure S1). We categorized each arsenic exposure variable, age, and BMI as tertiles for this purpose. We detected a difference only for a history of prior pregnancy loss; TTP was shorter among those women with a prior loss relative to those without (P = 0.03).

We generated multivariable Cox proportional hazard models to estimate the effects of residential drinking water arsenic exposure on TTP, among (n = 94) women with planned pregnancies. We used (log (−log S(t)) vs. (log TTP) graphs in a preliminary unadjusted assessment of the proportional hazards assumption for confounding variables identified by our DAG, including age, education, and cigarette smoking (Figure S2). We further investigated proportional hazards assumption violations in the multivariable context by incorporating log TTP cross-product terms with arsenic exposure variables, maternal age, or education, adjusted for age, education, and smoking; the arsenic exposure (P = 0.002), age (P < 0.0001), and education (P < 0.0001) interactions were statistically significant. Because the proportional hazards assumption was violated in models assessing arsenic exposure, maternal age, and education, we used stepwise backwards elimination to simultaneously evaluate arsenic exposure, maternal age, and education x log TTP cross-product terms, retaining only cross-products for arsenic exposure (P ≤ 0.02) and maternal age (P < 0.0001) in the final Cox-proportional hazards regression models, adjusted for maternal age, cigarette smoking, and education. Including the cross-product terms allowed for the effects of arsenic and maternal age to vary over time. We created a separate model for each arsenic exposure variable - average arsenic level, peak arsenic level, and daily arsenic dose. In all cases, higher levels of arsenic were associated with a lower probability for pregnancy among women with longer TTPs (Table 2). For example, relative to the likelihood for a first cycle pregnancy in an unexposed woman (i.e. average drinking water arsenic = 0 μg/L), a woman with average drinking water arsenic = 1.0 μg/L had a slightly higher risk for pregnancy in the 1st cycle (FHR = 1.08), yet a 5% lower risk in the 6th cycle given the joint effects of arsenic and time (FHR = 0.95), an 8% lower risk in the 9th cycle given the joint effects of arsenic and time (FHR = 0.92), and a 10% lower risk in the 12th cycle given the joint effects of arsenic and time (FHR = 0.90). We detected a similar pattern for more advanced maternal age.

The impacts of pregnancy planning and clinical infertility was of concern. To address the issue of left truncation associated with unplanned pregnancies, we included (n = 193) women with unplanned pregnancies in a sensitivity analysis (Table 3). Setting TTP = 0 for unplanned pregnancies, the average arsenic exposure similarly increased TTP, although to a

lesser degree than for planned pregnancies only. For example, relative to the likelihood for a 1st cycle pregnancy in women with 0 μg/L drinking water arsenic, women with exposure to 1 μg/L average drinking water arsenic had FHRs of 0.96 (4% lower risk), 0.94 (6% lower risk), and 0.94 (6% lower risk) for the joint effects of arsenic and time in the 6th, 9th, and 12th cycles, respectively. To evaluate potential bias due to clinical infertility we excluded n = 13 with TTP > 12 months in a second sensitivity analysis (Table 4). The effect of arsenic exposure on TTP was greater than for all planned pregnancies, as we detected even larger reductions in FHRs with longer TTPs.

4. Discussion

In this retrospective cohort study we report no main effects for low level drinking water arsenic contamination on women's fecundity, although we found modestly lower conditional pregnancy probabilities for women with higher arsenic exposures coupled to longer TTPs. The association between drinking water arsenic exposure and pregnancy among women with longer waiting times was consistent after adjustment for maternal age, education level, and cigarette smoking, and after conducting sensitivity analyses to assess the impacts of planning bias and clinical infertility. These data suggest that women with longer TTPs may be susceptible to arsenic-associated reproductive toxicity, even at low levels of exposure, although with no main effect. It is tempting to speculate that vulnerability to arsenic-associated reproductive toxicity is enhanced among less fecund women, those taking longer to conceive. To the best of our knowledge, this is the first human study to examine associations between drinking water arsenic exposure and female fecundity.

Our study examined drinking water arsenic exposures primarily in the 1 to 10 μg/L range, mostly at or below the 10 μg/L MCL recommended by the WHO, and enforced by the U.S. and the European Union (EU), including in Romania (Neamtiu et al., 2015; WHO, 2006). This limit, however, is predicated on cancer risk (ATSDR, 2007), and so may not be protective for reproductive endpoints. There has been very little experimental and observational research conducted to investigate the impact of arsenic exposure on female fecundity. An experimental study of pregnant mice exposed to 1, 10, 20, 37.5, 75, or 150 parts per million (ppm) arsenic in drinking water beginning at conception, reported a dose-dependent decrease in fecundity rate, defined as the product of the birth rate and litter size normalized to the unexposed control group, and a dose-dependent increase in the spontaneous abortion rate, beginning with the 20 ppm dose group (He et al., 2007). However, a prior observational study of women residing in western New York State and consuming high levels of sport fish reported no association between blood arsenic and TTP (Bloom et al., 2011). Yet, exposure in that study was believed to be primarily to non-toxic organic arsenic species, from the consumption of seafood. The parent case-control study for the current analysis reported a 1.75 higher odds (95% CI = 0.75–4.10) for spontaneous clinical pregnancy loss with 10 μg/L higher average drinking water arsenic, although among cigarette smokers only (Bloom et al., 2014a). The outcome in that study was clinically recognized loss, whereas our outcome in the current analysis was TTP as a measure of fecundity; together these studies suggest that low level drinking water arsenic contamination may be a reproductive toxicant for specific subpopulations of women. Unfortunately, the limited number of pregnancy planners who were also smokers (n = 38) in our study did not

allow for stratification in the context of adjustment for the aforementioned violations of the proportional hazards assumption (i.e., arsenic exposure and maternal age).

The results of this pilot study are limited by several important issues. The retrospective nature of our study design did not allow for collection of detailed information regarding ovulation and timing of intercourse. Women appear to have difficulty recalling TTP over long time intervals (Cooney et al., 2009); however, recall accuracy appears to be high within several months of a pregnancy (Zielhuis et al., 1992), and our data were collected within 4.5 months of conception. Furthermore, women in our study were unaware of their arsenic exposure and so any recall error is unlikely to have varied by TTP. Still, our retrospective study design included only clinically recognized pregnancies. This undersampling for sterile and subfecund couples (i.e., left truncation) may bias results if related to exposure, in particular if exposed women had conceived but suffered unrecognized early losses (Joffe et al., 2005); however, given the modest nature of the detected effect we do not believe there was a substantial impact. Yet, TTP data was available only for planned pregnancies, which may bias study results away from the null if behaviors associated with arsenic exposure differ from unplanned pregnancies (Weinberg et al., 1994). Our study sample included a high proportion of unplanned pregnancies, approximately 66% compared to approximately 50% on average in the U.S. (Finer and Zolna, 2011) However, given the nature of the Romanian health care system and dissimilarities in contraception practices, we do not believe this difference reflects a sampling bias, but rather a difference in cultural norms and health-related behaviors (Johnson et al., 2004). Accordingly, we found similar results when including unplanned pregnancies as TTP = 0, and so we believe any impact to have been minimal. We also excluded women reporting use of fertility services to conceive the study pregnancy and conducted a sensitivity analysis in which women who took more than 12 cycles to conceive a pregnancy were excluded, producing similar results.

Our exposure assessment strategy comprised environmental sampling coupled to questionnaire data. The absence of an exposure biomarker may have misclassified some women, given inter-individual variability in arsenic metabolism (Engstrom et al., 2009), and possible exposure sources in addition to residential drinking water, such as diet (EFSA, 2014). In an earlier study, we reported a modest correlation (r = 0.35) between inorganic urine arsenic and average drinking water arsenic levels for a subsample (n = 20) of our overall study population (Neamtiu et al., 2015). However, arsenic has a short half-life *in vivo*, approximately 10 hours, and so the impact of exposure misclassification is difficult to ascertain. We cannot rule out confounding by other reproductive toxicants that might track with drinking water arsenic and also impact fecundity. Air pollution, for example, has been linked to lower fecundity (Nieuwenhuijsen et al., 2014), and levels of CO and NO_x that exceeded EU air quality standards occurred within limited areas of Timişoara during 2008 (Popescu et al., 2011). Still, we used urban vs. rural residence as a proxy for air pollution exposure and there was little impact on the study results. Future studies should include a more comprehensive exposure assessment, using urinary biomarkers in addition to environmental sampling, in order to integrate arsenic exposure from all sources.

Reproduction is a couple-level function, yet we were only able to collect maternal exposure and covariate data. Evidence suggests that arsenic exposure may also impact male fecundity

(Meeker et al., 2008; Xu et al., 1993), and so the absence of paternal exposure data may have misclassified exposure. Still, more than 90% of our participants with planned pregnancies reported being married or living as married, thus similar residential drinking water arsenic exposures can be anticipated. We also generated similar effect estimates when including unplanned pregnancies as TTP = 0 conceptions in a sensitivity analysis, and so we suspect any impact to have been modest.

5. Conclusions

While the results of this pilot study are preliminary, they suggest that low level arsenic exposure through consumption of arsenic contaminated residential drinking water may impair fecundity among women with longer waiting times to pregnancy. Among less fecund couples, arsenic exposures, even at levels below 10 μg/L, might further impair fecundity.

Our study is an important first step to understanding the impact of low level drinking water arsenic exposure on female fecundity. These findings are particularly concerning given the widespread distribution of low level drinking water arsenic contamination worldwide, with millions of reproductively-aged women likely to be exposed. A future prospective study that includes home testing in order to capture ovulation, timing of intercourse, and pregnancy prior to clinical recognition, will allow for inclusion of sterile and subfecund couples, and longitudinal urine collection by the female and male partners will reduce exposure misclassification. Until more definitive data are available, the feasibility for use of alternate water sources might be considered by couples exposed to drinking water arsenic and who are experiencing difficulty conceiving a pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We assessed low level drinking water arsenic as a predictor of fecundability.
- Arsenic did not affect time to pregnancy among women conceiving quickly.
- Arsenic increased time to pregnancy among women taking longer to conceive.
- Low level drinking water arsenic may adversely impact women with lower fecundity.

Table 1

Demographic factors and drinking water arsenic exposures among planned pregnancies and unplanned pregnancies.

Factor	Planned pregnancies						Unplanned pregnancies						P-value [a]
	n	Mean	Median/%	SD	Min	Max	n	Mean	Median/%	SD	Min	Max	
Time to pregnancy (cycles)	94	8	3	13.5	1	72	-	-	-	-	-	-	-
Age (years)	94	29.57	28.96	5.01	18.50	42.17	193	28.78	28.08	6.28	18.08	44.25	0.20
BMI (kg/m^2)	93	22.92	22.21	3.74	16.14	36.73	192	22.28	21.20	3.89	15.06	46.61	0.07
Urban residence	68	--	72%	--	--	--	127	--	66%	--	--	--	0.28
Married, living as married	85	--	90%	--	--	--	152	--	79%	--	--	--	**0.02**
Education													
University diploma	55	--	59%	--	--	--	66	--	34%	--	--	--	**0.001**
High school diploma/some college/technical school	21	--	22%	--	--	--	71	--	37%	--	--	--	--
No high school diploma	18	--	19%	--	--	--	56	--	29%	--	--	--	--
Smoked > 100 lifetime cigarettes	38	--	40%	--	--	--	74	--	38%	--	--	--	0.80
Reproductive history													
Prior pregnancies	60	--	64%	--	0	6	109	--	56%	--	0	14	0.79
Prior pregnancy losses	48	--	51%	--	0	3	74	--	38%	--	0	8	0.09
History of PID	16	--	17%	--	--	--	30	--	16%	--	--	--	0.86
Drinking water arsenic level													
Average (μg/L)	94	4.63	0.46	18.79	0	175.10	193	4.11	0.29	12.19	0	130.30	0.80
Peak (μg/L)	94	5.55	1.11	19.04	0	175.10	193	4.59	0.53	12.44	0	130.30	0.56
Daily dose (μg)	94	7.29	0.77	36.42	0	350.20	193	5.75	0.44	13.44	0	90.45	0.45

NOTE: Bold typeface indicates $P < 0.05$.

[a] Wilcoxon rank sum tests test for continuous variables and chi-square test for categorical variables.

Table 2

Fecundability hazard ratios (FHR) and 95% confidence intervals (CI) for time to pregnancy (TTP) associated with drinking water arsenic exposure (n = 94).

Predictor [a]	FHR	95% CI	P-value
Average arsenic level (μg/L)	1.08	1.02, 1.13	**0.01**
Maternal age (years)	1.34	1.25, 1.44	< **0.0001**
High school diploma/some [b] college/technical school	1.17	0.56, 2.46	0.68
University diploma [b]	1.28	0.65, 2.51	0.47
Smoked > 100 lifetime cigarettes [c]	0.93	0.56, 1.55	0.78
Maternal age (years) x log TTP (cycles)	0.80	0.77, 0.84	< **0.0001**
Average arsenic level (μg/L) x log TTP (cycles)	0.93	0.89, 0.98	**0.01**
Peak arsenic level (μg/L)	1.06	1.01, 1.11	**0.02**
Maternal age (years)	1.34	1.25, 1.44	< **0.0001**
High school diploma/some college/technical school [b]	1.14	0.54, 2.41	0.74
University diploma [b]	1.35	0.68, 2.65	0.39
Smoked > 100 lifetime cigarettes [c]	0.97	0.58, 1.61	0.90
Maternal age (years) x log TTP (cycles)	0.81	0.77, 0.84	< **0.0001**
Peak arsenic level (μg/L) x log TTP (cycles)	0.95	0.91, 0.99	**0.02**
Daily arsenic dose (μg)	1.06	1.01, 1.11	**0.01**
Maternal age (years)	1.34	1.24, 1.44	< **0.0001**
High school diploma/some college/technical school [b]	1.12	0.53, 2.38	0.77
University diploma [b]	1.33	0.67, 2.63	0.42
Smoked > 100 lifetime cigarettes [c]	0.98	0.59, 1.63	0.95
Maternal age (years) x log TTP (cycles)	0.81	0.77, 0.84	< **0.0001**
Daily arsenic dose (μg) x log TTP (cycles)	0.95	0.91, 0.99	**0.02**

NOTE: Bold typeface indicates P < 0.05. FHR > 1.0 indicates a higher conditional likelihood for pregnancy and FHR < 1.0 indicates a lower conditional likelihood for pregnancy.

[a] Effect estimates adjusted for all other variables in the table;

[b] 'No high school diploma' is reference category;

[c] 'Smoked < 100 lifetime cigarettes' is reference category.

Table 3

Fecundability hazard ratios (FHR) and 95% confidence intervals (CI) for time to pregnancy (TTP) associated with drinking water arsenic exposure among planned and unplanned pregnancies (n = 287).

Predictor [a]	**FHR**	**95% CI**	**P-value**
Average arsenic level (μg/L)	1.01	1.00, 1.01	0.30
Maternal age (years)	1.04	1.02, 1.06	**0.0004**
High school diploma/some college/technical school [b]	0.96	0.69, 1.33	0.79
University diploma [b]	1.07	0.78, 1.47	0.68
Smoked > 100 lifetime cigarettes [c]	0.91	0.71, 1.17	0.45
Maternal age (years) x log TTP (cycles)	0.87	0.85, 0.98	< **0.0001**
Average arsenic level (μg/L) x log TTP (cycles)	0.97	0.94, 0.98	**0.01**

NOTE: Bold typeface indicates P < 0.05. FHR > 1.0 indicates a higher conditional likelihood for pregnancy and FHR < 1.0 indicates a lower conditional likelihood for pregnancy.

[a] Effect estimates adjusted for all other variables in the table;

[b] 'No high school diploma' is reference category;

[c] 'Smoked < 100 lifetime cigarettes' is reference category.

Table 4

Fecundability hazard ratios (FHR) and 95% confidence intervals (CI) for time to pregnancy (TTP) associated with drinking water arsenic exposure among planned pregnancies with TTP ≤ 12 cycles (n = 82).

Predictor [a]	**FHR**	**95% CI**	**P-value**
Average arsenic level (μg/L)	1.12	1.06, 1.19	< **0.0001**
Maternal age (years)	1.34	1.23, 1.45	< **0.0001**
High school diploma/some college/technical school [b]	0.84	0.39, 1.77	0.64
University diploma [b]	0.94	0.49, 1.79	0.85
Smoked > 100 lifetime cigarettes [c]	0.76	0.44, 1.31	0.32
Maternal age (years) x log TTP (cycles)	0.79	0.75, 0.83	< **0.0001**
Average arsenic level (μg/L) x log TTP (cycles)	0.89	0.83, 0.94	**0.0001**

NOTE: Bold typeface indicates P < 0.05. FHR > 1.0 indicates a higher conditional likelihood for pregnancy and FHR < 1.0 indicates a lower conditional likelihood for pregnancy.

[a] Effect estimates adjusted for all other variables in the table;

[b] 'No high school diploma' is reference category;

[c] 'Smoked < 100 lifetime cigarettes' is reference category.