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ORIGINAL ARTICLE *Infertility*

Urinary bisphenol A concentrations and association with *in vitro* fertilization outcomes among women from a fertility clinic

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STUDY QUESTION: Are urinary BPA concentrations associated with *in vitro* fertilization (IVF) outcomes among women attending an academic fertility center?

SUMMARY ANSWER: Urinary BPA concentrations were not associated with adverse reproductive and pregnancy outcomes among women from a fertility clinic.

WHAT IS KNOWN ALREADY: Bisphenol A (BPA), an endocrine disruptor, is detected in the urine of most Americans. Although animal studies have demonstrated that BPA reduces female fertility through effects on the ovarian follicle and uterus, data from human populations are scarce and equivocal.

STUDY DESIGN, SIZE AND DURATION: This prospective cohort study between 2004 and 2012 at the Massachusetts General Hospital Fertility Center included 256 women ($n = 375$ IVF cycles) who provided up to two urine samples prior to oocyte retrieval (total $N = 673$).

PARTICIPANTS/MATERIALS, SETTINGS, METHODS: Study participants were women enrolled in the Environment and Reproductive Health (EARTH) Study. Intermediate and clinical end-points of IVF treatments were abstracted from electronic medical records. We used generalized linear mixed models with random intercepts to evaluate the association between urinary BPA concentrations and IVF outcomes adjusted by age, race, body mass index, smoking status and infertility diagnosis.

MAIN RESULTS AND THE ROLE OF CHANCE: The specific gravity-adjusted geometric mean of BPA was 1.87 μg/l, which is comparable to that for female participants in the National Health and Nutrition Examination Survey, 2011–2012. Urinary BPA concentrations were not associated with endometrial wall thickness, peak estradiol levels, proportion of high quality embryos or fertilization rates. Furthermore, there were no associations between urinary BPA concentrations and implantation, clinical pregnancy or live birth rates per initiated cycle or per embryo transfer. Although we did not find any associations between urinary BPA concentrations and IVF outcomes, the relation between BPA and endometrial wall thickness was modified by age. Younger women (<37 years old) had thicker endometrial thickness across increasing quartiles of urinary BPA concentrations, while older women (≥37 years old) had thinner endometrial thickness across increasing quartiles of urinary BPA concentrations.

LIMITATIONS, REASONS FOR CAUTION: Limitations to this study include a possible misclassification of BPA exposure and difficulties in extrapolating the findings to the general population.

WIDER IMPLICATIONS OF THE FINDINGS: Data on the relation between urinary BPA concentrations and reproductive outcomes remain scarce and additional research is needed to clarify its role in human reproduction.

STUDY FUNDING/COMPETING INTEREST(S): This work was supported by NIH grants R01ES022955, R01ES009718 and R01ES000002 from the National Institute of Environmental Health Sciences (NIEHS) and grant T32DK00770316 from the National Institute of Child Health and Human Development (NICHD). None of the authors has any conflicts of interest to declare. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Key words: bisphenol A / IVF outcomes / epidemiology / reproductive health / endocrine disruptor

Introduction

Bisphenol A (BPA) is a high production volume chemical that has received substantial scientific and regulatory attention over the past decade. BPA is widely used in the manufacture of a variety of consumer products such as polycarbonate plastics, epoxy resin liners of canned foods, some dental sealants and composites, and thermal receipts (Ehrlich *et al.*, 2014). BPA was detected in over 90% of urine samples obtained from participants in the 2003–2004 and 2011–2012 National Health and Nutrition Examination Survey (NHANES) (Calafat *et al.*, 2008; Centers for Disease Control and Prevention (CDC), 2015), showing that there is widespread general population exposure (Vandenberg *et al.*, 2007). Aglycone (unconjugated) BPA has weak estrogenic activity through binding with estrogen receptors α and β (Gould *et al.*, 1998, Kuiper *et al.*, 1998). In addition, aglycone BPA has high affinity for two membrane-bound estrogen receptors, G protein-coupled estrogen receptor 30 (GPR30) (Dong *et al.*, 2011) and membrane estrogen receptor alpha (mERα) (Wozniak *et al.*, 2005), as well as for an orphan nuclear estrogen-related receptor gamma (ERRγ) (Matsushima *et al.*, 2007, Okada *et al.*, 2008). BPA has also been shown in experimental animal studies to bind to the androgen receptor, peroxisome proliferator-activated receptor γ, and thyroid hormone receptor (Richter *et al.*, 2007).

These endocrine activities of BPA have been shown to lead to adverse reproductive outcomes in animal models. Animal data have shown that BPA primarily affects female fertility through its effects on the ovarian follicle and uterus. For example, BPA adversely affects oocyte meiosis (Hunt *et al.*, 2003; Brieno-Enriquez *et al.*, 2011), interferes with germ cell nest breakdown (Rivera *et al.*, 2011), reduces the primordial follicle pool by stimulating their initial recruitment and subsequent follicle development until the antral stage (Rivera *et al.*, 2011), alters ovarian steroidogenesis (Fernandez *et al.*, 2010; Xi *et al.*, 2011), modifies normal uterine morphology (Berger *et al.*, 2010), and impairs uterine receptivity and ova-implantation (Berger *et al.*, 2007, 2008; Crawford and Decatanzaro, 2012). There is limited evidence on the effect of BPA on pregnancy outcomes. Low-dose BPA exposure (<1600 μg/day) has reduced the number of live pups born in exposed CD1 mice (Cabaton *et al.*, 2011) and Holtzman rats (Salian *et al.*, 2009b). Moreover, it has decreased the number of total pups born to unexposed female Holtzman rats mated to neonatally and gestationally exposed males of the same strain (Salian *et al.*, 2009a,b). However, other studies in mice and rats have shown that higher-dose BPA exposure (up to 50 mg/day) is not associated with the number of live pups or total number of delivered pups (Howdeshell *et al.*, 2008; Tyl *et al.*, 2008; Thuillier *et al.*, 2009; Kobayashi *et al.*, 2010, 2012; Ryan *et al.*, 2010; Xi *et al.*, 2011; Nanjappa *et al.*, 2012).

Several studies have investigated the impact of BPA on female reproductive and pregnancy outcomes (Galloway *et al.*, 2010; Mok-Lin *et al.*, 2010; Bloom *et al.*, 2011a,b; Fujimoto *et al.*, 2011; Ehrlich *et al.*, 2012a,b). We previously reported that in women undergoing *in vitro* fertilization (IVF), urinary BPA concentrations were inversely associated with peak serum estradiol levels, number of oocytes at retrieval (overall and mature), and number of normally fertilized oocytes (Mok-Lin *et al.*, 2010; Ehrlich *et al.*, 2012b). We also reported that higher urinary BPA concentrations were associated with a suggestive decrease in trend of implantation (Ehrlich *et al.*, 2012a). The objective of the current analysis was to reevaluate, in a much larger number of women from the same cohort (Mok-Lin *et al.*, 2010; Ehrlich *et al.*, 2012a,b), the associations of urinary BPA concentrations with early IVF outcomes (i.e. peak estradiol, oocyte yield, fertilization rate). In addition, we expand on our previous research (Mok-Lin *et al.*, 2010; Ehrlich *et al.*, 2012a,b) by exploring the potential relationships of urinary BPA concentrations with clinical pregnancy and live birth outcomes.

Materials and Methods

Study population

Study participants were women enrolled in the Environment and Reproductive Health (EARTH) Study, an ongoing prospective cohort established to evaluate environmental and dietary determinants of fertility (Hauser *et al.*, 2006). Women between 18 and 45 years old were eligible to participate and ~60% of those contacted by the research nurses enrolled. The current analysis includes 256 women who completed at least one IVF cycle between November 2004 and April 2012 ($n = 375$ cycles) at the Massachusetts General Hospital (MGH) Fertility Center, and had provided at least one urine sample for the measurement of BPA per IVF cycle. IVF cycles in which women used an oocyte donor ($n = 18$) or cryopreservation-thaw cycles ($n = 34$) were excluded from the present study. The study was approved by the Human Studies Institutional Review Boards of the MGH, Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC). Participants signed an informed consent after the study procedures were explained by a research nurse and all questions were answered.

Clinical management and assessment of outcomes

Clinical information was abstracted from the patient's electronic medical record by research staff. Follicle stimulating hormone (FSH) and estradiol concentrations were measured in blood serum, collected on the third day of the menstrual cycle, using an automated electrochemiluminescence immunoassay at the MGH Core Laboratory as previously described (Mok-Lin

et al., 2010). The peak estradiol concentration was defined as the highest level of estradiol prior to oocyte retrieval, which was obtained on the day of trigger with hCG. Subsequent to an infertility evaluation, each patient was assigned an infertility diagnosis by a physician at the MGH Fertility Center according to the Society for Assisted Reproductive Technology (SART) definitions as previously described (Mok-Lin et al., 2010). The participant's date of birth was collected at entry, and weight and height were measured by the nurse. Body mass index (BMI) was calculated as weight (in kilograms) per height (in meters) squared.

Women underwent one of three controlled ovarian stimulation IVF treatment protocols on Day 3 of induced menses after completing a cycle of oral contraceptives: (i) luteal phase GnRH-agonist protocol, (ii) follicular phase GnRH-agonist/Flare protocol or (iii) GnRH-antagonist protocol. Lupron dose was reduced at, or shortly after, the start of ovarian stimulation with FSH/hMG in the luteal phase GnRH-agonist protocol. FSH/hMG and GnRH-agonist or GnRH-antagonist was continued to the day of trigger with human chorionic gonadotrophin (hCG). Throughout the monitoring phase of the subject's IVF treatment cycle, estradiol levels were obtained (Elecsys Estradiol II reagent kit, Roche Diagnostics). Oocyte retrieval was completed when follicle dimensions on transvaginal ultrasound reached 16–18 mm and the estradiol level reached at least 500 pg/ml. Patients were monitored during gonadotrophin stimulation for serum estradiol, follicle size measurements and counts, and endometrial thickness through to 2 days before oocyte retrieval. Human chorionic gonadotrophin (hCG) was administered ~36 h before the scheduled oocyte retrieval procedure to induce ovulation. Details of the oocyte retrieval have been previously described (Mok-Lin et al., 2010).

Embryologists determined the total number of oocytes retrieved per cycle and classified them as germinal vesicle, metaphase I, metaphase II (MII) or degenerated. Oocytes underwent either conventional IVF or intracytoplasmic sperm injection (ICSI) as clinically indicated. Embryologists determined the fertilization rate 17–20 h after insemination as the number of oocytes with two pronuclei divided by the number of MII oocytes inseminated. We classified embryo quality based on morphology and number of blastomeres, ranging from 1 (best) to 5 (worst) on Day 2 and 3. For analysis, we classified embryos as best quality if they had 4 cells on Day 2, 8 cells on Day 3, and a morphologic quality score of 1 or 2 on Days 2 and 3 (Veeck and Zaninovic, 2003). An overall score of 1 or 2 was considered high quality, 3 was considered intermediate quality and 4 or 5 indicated poor quality embryos.

In women who underwent an embryo transfer, clinical outcomes were assessed. Implantation was defined as a serum β-hCG level >6 mIU/ml, typically measured 17 days (range 15–20 days) after oocyte retrieval. An elevation in β-hCG with the confirmation of an intrauterine pregnancy on an ultrasound at 6 weeks was considered a clinical pregnancy. A live birth was defined as the birth of a neonate on or after 24 weeks gestation.

Urine sample collection and BPA measurements

Women provided up to two spot urine samples per IVF cycle, with the first one (not necessarily a fasting sample) collected between Day 3 and Day 9 of the gonadotrophin phase, and the second one, always a fasting sample, collected on the day of oocyte retrieval, prior to the procedure or administration of intravenous fluids. Urine was collected in a sterile, clean polypropylene specimen cup at the MGH Fertility Center. Specific gravity (SG), which was used to correct BPA concentrations for urine dilution, was measured at room temperature using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA) calibrated with deionized water before each measurement within an hour of the urine being produced. The urine was then divided into aliquots, frozen and stored at −80°C. Samples were shipped on dry ice overnight to the CDC where they were stored at or below −40°C until analysis. The urinary concentrations of the sum of free and conjugated BPA species (total BPA) were measured using online solid-phase extraction (SPE) coupled with isotope dilution-high-performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS), as described before (Ye et al., 2005). First, 100 μl of urine was treated with β-glucuronidase/sulfatase (Helix pomatia, H1; Sigma Chemical Co, St. Louis, MO, USA) to hydrolyze the BPA-conjugated species. BPA was then retained and concentrated on a C18 reversed-phase size-exclusion SPE column (Merck KGaA, Germany), separated from other urine matrix components using a pair of monolithic HPLC columns (Merck KGaA), and detected by negative ion-atmospheric pressure chemical ionization-MS/MS. The limit of detection (LOD) was 0.4 μg/l. In addition to study samples, each analytical run included low-concentration and high-concentration quality control materials, prepared with spiked pooled human urine, and reagent blanks to assure the accuracy and reliability of the data (Ye et al., 2005). BPA concentrations were adjusted for dilution using the following formula: $Pc = P[(1.015 - 1)/SG - 1]$, where Pc is the SG-corrected BPA concentration (μg/l), P is the measured BPA concentration (μg/l) of the urine sample, and 1.015 is the mean SG concentration in the study population (Smith et al., 2012). The geometric mean of the SG-adjusted BPA concentrations from two spot urine samples collected during each IVF cycle was used as a measure of cycle-specific urinary BPA concentration. For cycles with only one urine sample (~20%), the BPA concentration for that single urine sample was used as the cycle-specific urinary BPA concentration. BPA concentrations below the LOD were assigned a value equal to the LOD divided by the square root of 2 prior to SG adjustment as described previously (Meeker et al., 2010).

Statistical analysis

Demographic and baseline reproductive characteristics of the women are presented using median ± interquartile ranges (IQRs) or percentages. Women's exposures to BPA were categorized into quartiles of urinary BPA concentrations (based on the woman's cycle-specific SG-adjusted geometric mean of BPA as described above) with the lowest quartile considered as the reference group. Associations between urinary BPA concentrations and demographics and baseline reproductive characteristics were evaluated using Kruskal–Wallis tests for continuous variables and chi-squared tests for categorical variables (data not shown). Multivariable generalized linear mixed models with random intercepts were used to evaluate the association between urinary BPA concentrations and IVF outcomes. A Poisson distribution and log link function were specified for oocyte counts, a normal distribution and identity link function were specified for endometrial wall thickness and E2 trigger levels, and a binomial distribution and logit link function were specified for embryo quality, fertilization rates, and clinical outcomes (implantation, clinical pregnancy and live birth). To explore whether associations between urinary BPA concentrations and IVF outcomes were modified by age and insemination method, product cross-product term of quartiles of urinary BPA concentrations and the modifier (both as ordinals variables) was entered into the model. Tests for linear trends (Rosner, 2000) were conducted using the median values of each quartile of urinary BPA concentration as a continuous variable. To allow for better interpretation of the results, population marginal means (Searle et al., 1980) are presented which accounts for all the covariates in the model.

Confounding was assessed using prior knowledge on biological relevance and descriptive statistics from our study population through the use of directed acyclic graphs (Weng et al., 2009). The variables considered as potential confounders included factors previously related to IVF outcomes in this and other studies, and factors associated with BPA exposure and IVF outcomes in this study, regardless of whether they had been previously described as predictors of IVF outcomes (Table I). Because collection of the samples included in this analysis spanned 8 years, and during this period urinary BPA concentrations and IVF success rates may have changed, a variable for calendar

Table I Baseline characteristics of 256 women in the Environment and Reproductive Health Study (EARTH) by quartiles of specific gravity adjusted urinary BPA concentrations (μg/l).

	Total cohort Median (IQR) or *N* (%)	Q1 Median (IQR) or *N* (%)	Q4	*P*, value[a]
Baseline characteristics				
Age, years	35.0 (32.5, 39.0)	36.0 (33.0, 39.0)	35.0 (32.0, 39.0)	0.44
Race/ethnic group, *n* (%)				
White/Caucasian	211 (82.4)	60 (74.1)	35 (87.5)	0.06
Black	6 (2.4)	1 (1.2)	3 (7.5)	
Asian	21 (8.2)	12 (14.8)	1 (2.5)	
Other	18 (7.0)	8 (9.9)	1 (2.5)	
Body mass index, kg/m^2	23.0 (21.0, 26.0)	23.0 (21.5, 25.9)	22.5 (21.3, 24.4)	0.62
Ever smoker, *n* (%)	71 (27.7)	19 (23.5)	13 (32.5)	0.70
Education, *n* (%)				0.86
< College graduate	20 (7.8)	5 (6.6)	4 (10.5)	
College graduate	77 (30.1)	21 (28.0)	13 (34.2)	
Graduate degree	140 (59.1)	49 (65.3)	21 (55.3)	
Baseline reproductive characteristics				
Previous IUI, *n* (%)	120 (46.7)	33 (40.7)	16 (40.0)	0.27
Previous IVF, *n* (%)	61 (23.8)	24 (29.6)	9 (22.5)	0.03
Initial infertility diagnosis, *n* (%)				0.77
Male factor	94 (36.7)	32 (39.5)	17 (42.5)	
Female factor	78 (30.5)	22 (27.2)	14 (35.0)	
Diminished ovarian reserve	18 (7.0)	4 (4.9)	3 (7.5)	
Endometriosis	18 (7.0)	6 (7.4)	3 (7.5)	
Ovulation disorders	22 (8.6)	4 (4.9)	5 (12.5)	
Tubal	18 (7.1)	6 (7.4)	3 (7.5)	
Uterine	2 (0.8)	2 (2.4)	0 (0)	
Unexplained	84 (32.8)	27 (33.3)	9 (22.5)	
Initial treatment protocol, *n* (%)				
Antagonist	29 (11.3)	12 (14.8)	7 (17.5)	0.51
Flare[b]	41 (16.0)	13 (16.1)	7 (17.5)	
Luteal phase agonist[c]	186 (72.3)	56 (69.1)	26 (65.0)	
Initial ICSI cycles, *n* (%)	130 (53.3)	44 (55.7)	21 (53.8)	0.83
E2 trigger levels, pmol/l	2017.0 (1453.0, 2639.0)	2100.0 (1530.0, 2540.5)	1773.0 (1325.0, 2630.0)	0.39
Day 3 FSH levels, IU/ l	6.9 (5.8, 8.3)	6.8 (5.6, 8.5)	7.4 (6.3, 8.2)	0.17
Embryo transfer day, *n* (%)				
No embryos transferred	27 (10.5)	8 (9.9)	1 (2.5)	
Day 2	13 (5.7)	3 (4.1)	1 (2.6)	0.52
Day 3	134 (55.5)	48 (65.8)	25 (64.1)	
Day 5	82 (35.8)	22 (30.1)	13 (33.3)	
Number of embryos transferred, *n* (%)				
No embryos transferred	27 (10.5)	8 (9.9)	1 (2.5)	0.09
1 embryo	29 (11.3)	9 (11.1)	6 (15.0)	
2 embryos	150 (58.6)	44 (54.3)	27 (67.5)	
3+ embryos	47 (18.6)	20 (24.7)	6 (15.0)	

BPA, bisphenol A; IQR, interquartile range; *N*, number; IUI, intrauterine insemination, IVF, *in vitro* fertilization, ICSI, intracytoplasmic sperm injection.
[a]From Kruskal–Wallis test for continuous variables and χ^2 tests for categorical variables.
[b]Follicular phase GnRH-agonist/Flare protocol.
[c]Luteal phase GnRH-agonist protocol.

year was considered but not retained in the models because the estimates remained very similar with and without inclusion of calendar year. Final models were adjusted for age (continuous), BMI (continuous), race (white versus nonwhite), smoking status (never versus ever) and infertility diagnosis (male factor, female factor, unexplained). All tests were two-tailed and the level of statistical significance was set at 0.05. Statistical analyses were performed with SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

Results

The 256 women included in this analysis were predominantly Caucasian (82%) and the majority (71%) had never smoked (Table I). These women had a median age of 35 years (IQR: 33–39) and a median BMI of 23.0 kg/m^2 (IQR: 21.0–26.0). The primary SART diagnosis at enrollment was male factor (37%) followed by unexplained infertility (33%) and female factor infertility (31%) (ovulation disorders were the most common followed by tubal disorders, diminished ovarian reserve, endometriosis, and uterine disorders). Luteal phase GnRH-agonist protocols were the most commonly used stimulation protocol in the first treatment cycle (72%). Women had a median day 3 FSH of 6.9 IU/l. Caucasian and African-American women had higher urinary BPA concentrations as compared with Asian women (P, trend = 0.06). No other baseline characteristics were significantly related to urinary BPA concentrations.

The geometric mean of the 673 SG-adjusted urine samples provided by the 256 women (contributing to 375 IVF cycles) was 1.87 μg/l (Table II), comparable to that for females in NHANES 2009–2010 and 2011–2012 (2.09 and 1.91 μg/l, respectively) (Centers for Disease Control and Prevention (CDC), 2015) and similar to our earlier analysis (2.56 μg/l) (Ehrlich *et al.*, 2012a). Two urine samples were collected in 80% (299/375) of the IVF cycles. Detectable concentrations of BPA were measured in 88.7% (604/673) of urine samples (Table II).

The associations of urinary BPA concentrations with endometrial thickness and ovarian stimulation outcomes are shown in Table III. Although the overall trend was not statistically significant in adjusted models, women with urinary BPA concentrations in the highest quartile had slightly fewer total oocytes (n = 9.9) and MII oocytes (n = 8.3) compared with women in the lowest quartile (N = 10.9, P = 0.13; and N = 9.1, P = 0.21, respectively). Urinary BPA concentrations were not related to endometrial wall thickness or to peak estradiol levels. Similarly, urinary BPA concentrations were not associated with the proportion of high-quality embryos or the fertilization rates, either overall (Table III) or when IVF and ICSI cycles were examined separately (data not shown).

No significant dose–response associations were observed between urinary BPA concentrations and implantation, clinical pregnancy and live birth rates per initiated cycle in models adjusted for age, BMI, race/ethnicity, smoking status and primary infertility diagnosis (Fig. 1). The adjusted differences (95% confidence interval (CI)) in implantation, clinical pregnancy and live birth rates for women in the highest quartile of urinary BPA concentration compared with women in the lowest quartile were −0.02 (−0.13 to 0.09), −0.06 (−0.17 to 0.05) and −0.03 (−0.13 to 0.08), respectively. Because infertility diagnosis might be related to earlier BPA exposure and thus may be an intermediate on the causal pathway to IVF outcome, we conducted a sensitivity analysis removing infertility diagnosis and the results remained non-significant. Moreover, we performed analyses using urinary BPA concentrations as a continuous variable and there were no associations with any of the IVF outcomes (data not shown).

In order to facilitate comparisons of our results with our earlier publication based on a smaller subset of women from the same cohort, sensitivity analyses were carried out exploring the relation between urinary BPA concentrations and implantation per embryo transferred (Supplementary Table SI). We found no associations with these outcomes with the expanded population and extended follow-up. We also analyzed our data using a median of SG = 1.024 based on previous literature (Boeniger *et al.*, 1993; Teass *et al.*, 1993) and used in our earlier publication (rather than 1.015 used in the present analysis) and adjusted for the same set of covariates used in the previous publication (protocol type, Day 3 FSH result and number of embryo transferred) (Supplementary Table SI). We observed no association of BPA with infertility treatment outcomes in these supplementary analyses.

In evaluating whether effects of BPA depended on other factors, we observed significant effect modification by age in the association between BPA and endometrial wall thickness (P, interaction = 0.02). The adjusted differences (95% CI) in endometrial wall thickness for women with urinary BPA concentrations in the top quartile, compared with women in the bottom quartile, were +1.07 (+0.20, +1.30) among younger (P, trend = 0.06) and −0.60 (−1.30, +0.10) among older women (P, trend = 0.08). There was no evidence of significant heterogeneity of the relation between urinary BPA concentrations and other preclinical and clinical outcomes (P, interactions >0.1) by age.

Discussion

We evaluated the association of urinary concentrations of BPA with IVF outcomes among 256 women (n = 375 IVF cycles) attending a fertility

Table II Distribution of cycle-specific geometric mean of urinary BPA concentrations (μg/l) among 256 women in the Environment and Reproductive Health Study (EARTH) undergoing 375 IVF cycles (673 urine samples).

			Percentile							
	Detection rate	GM (SD)	Min	10th	25th	50th	75th	90th	95th	Max
BPA	88.7%	2.06 (2.20)	<LOD	0.57	0.89	1.47	2.40	3.87	5.48	22.07
SG-adj BPA		1.87 (1.57)	<LOD	0.74	0.97	1.38	2.24	3.50	4.87	16.55

All values below LOD were assigned a value equal to the LOD divided by √2. Seven (1.9%) cycle-specific BPA concentrations were < LOD and are included in the percentiles. There were 69 (10.3%) individual urine samples which had BPA concentrations < LOD. There was an 88.7% detection rate for all individual urine samples [SG range (1.001–1.038)].
< LOD, below limit of detection (0.4 μg/l); Max, maximum; Min, minimum; SG-adj BPA, specific-gravity adjusted BPA concentrations.

Table III Specific gravity adjusted urinary bisphenol A concentrations in relation to ovarian stimulation and endometrial thickness outcomes among 256 women in the Environment and Reproductive Health Study (EARTH) contributing to 375 fresh IVF cycles from an infertility clinic.

	Total oocyte yield, *n*		MII oocyte yield, *n*	
Urinary BPA concentrations (μg/l)	**Unadjusted**	**Adjusted[a]**	**Unadjusted**	**Adjusted[a]**
Q1 [<LOD–0.96]	11.0 (10.0, 12.1)	10.9 (9.9, 12.0)	9.2 (8.3, 10.1)	9.1 (8.2, 10.0)
Q2 [0.97–1.37]	11.0 (10.0, 12.1)	10.8 (9.9, 11.9)	9.1 (8.2, 10.0)	9.0 (8.1, 9.9)
Q3 [1.38–2.20]	11.1 (10.1, 12.3)	10.9 (9.9, 12.0)	9.8 (8.9, 10.8)	9.6 (8.7, 10.6)
Q4 [2.24–16.55]	9.9 (10.0, 11.0)	9.9 (8.9, 11.0)	8.3 (7.5, 9.3)	8.4 (7.6, 9.3)
P, trend[b]	0.09	0.13	0.13	0.21
	Endometrial wall thickness, mm		**E2 trigger levels, pmol/l**	
Urinary BPA concentrations (μg/l)	**Unadjusted**	**Adjusted[a]**	**Unadjusted**	**Adjusted[a]**
Q1 [<LOD–0.96]	9.9 (9.5, 10.3)	9.9 (9.5, 10.3)	2147.5 (1971.7, 2323.4)	2112.9 (1938.3, 2287.4)
Q2 [0.97–1.37]	10.2 (9.8, 10.6)	10.2 (9.8, 10.6)	2149.9 (1973.0, 2326.8)	2132.2 (1957.3, 2306.9)
Q3 [1.38–2.20]	10.6 (10.2, 11.0)	10.6 (10.2, 11.0)	2099.9 (1923.9, 2275.8)	2098.2 (1923.8, 2272.6)
Q4 [2.24–16.55]	10.1 (9.7, 10.6)	10.2 (9.7, 10.6)	1999.5 (1818.6, 2180.1)	2009.3 (1829.9, 2188.7)
P, trend[b]	0.76	0.63	0.17	0.31
	>1 Best embryo quality[c], proportion		**Fertilization, rate**	
Urinary BPA concentrations (μg/l)	**Unadjusted**	**Adjusted[a]**	**Unadjusted**	**Adjusted[a]**
Q1 [<LOD–0.96]	0.38 (0.28, 0.49)	0.39 (0.28, 0.50)	0.72 (0.68, 0.76)	0.72 (0.68, 0.77)
Q2 [0.97–1.37]	0.47 (0.36, 0.57)	0.47 (0.36, 0.58)	0.69 (0.64, 0.73)	0.69 (0.64, 0.73)
Q3 [1.38–2.20]	0.44 (0.33, 0.55)	0.43 (0.33, 0.54)	0.70 (0.65, 0.74)	0.70 (0.65, 0.74)
Q4 [2.24–16.55]	0.45 (0.35, 0.56)	0.45 (0.34, 0.56)	0.73 (0.68, 0.77)	0.73 (0.68, 0.77)
P, trend[b]	0.52	0.61	0.44	0.51

[a]Data are presented as predicted marginal means (95% CI) adjusted for age (continuous), BMI (continuous), smoking status (never and ever), race (white and others) and infertility diagnosis (male, female and unexplained).
[b]Tests for trend were performed using the median concentration of urinary bisphenol A in each group as a continuous variable in the model.
[c]We classified embryos as best quality if they had 4 cells on Day 2, 8 cells on Day 3, and a morphologic quality score of 1 or 2 on Days 2 and 3.

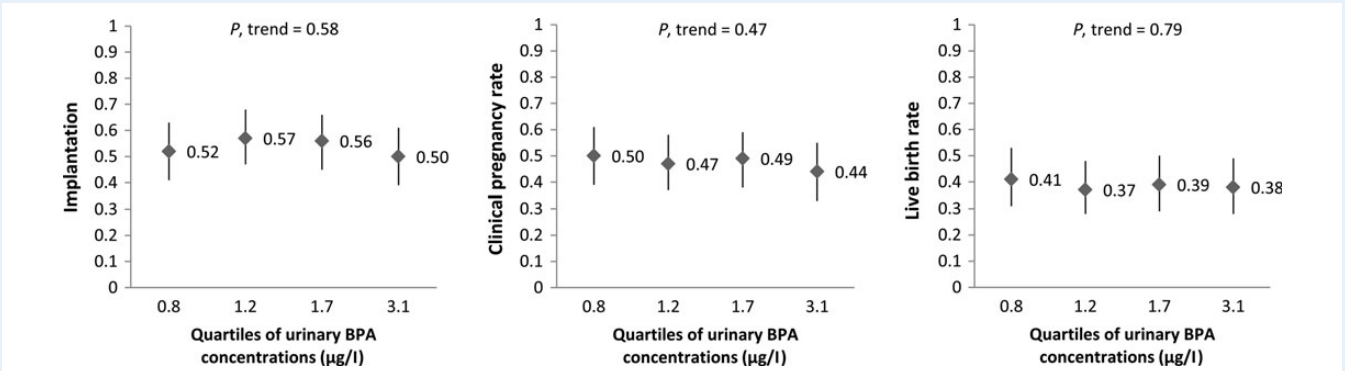

Figure 1 Adjusted rates (95% CI) in clinical outcomes per initiated cycle according to specific gravity adjusted urinary BPA concentrations (μg/l). Models are adjusted for age (continuous), BMI (continuous), smoking status (never and ever), race (white and others) and infertility diagnosis (male, female and unexplained). Tests for trend were performed using the median concentration of urinary BPA in each quartile as a continuous variable in the model. Implantation was defined as a serum β-hCG level >6 mIU/ml typically measured 17 days (range 15–20 days) after oocyte retrieval, clinical pregnancy as the presence of an intrauterine pregnancy confirmed by ultrasound and live birth as the birth of a neonate on or after 24 weeks gestation. Medians of each quartile of urinary BPA concentrations (μg/l) are presented.

clinic at the Massachusetts General Hospital and compared our results with our earlier publications on a smaller group of women from the same cohort (peak estradiol among 84 women and 112 cycles, implantation among 137 women and 180 cycles, and response to ovarian stimulation among 174 women and 237 cycles) (Mok-Lin *et al.*, 2010; Ehrlich *et al.*, 2012a,b). We also expanded upon our previous publications by exploring the relationships of urinary BPA concentrations with clinical pregnancy and live birth rates. In the present analysis, we found no associations of urinary BPA concentrations with measures of ovarian stimulation (oocyte yield), endometrial thickness, embryo quality, fertilization rates, implantation, clinical pregnancy and live birth rates.

Although the results for total and MII oocyte yield were not significant in our current analyses, they were in similar directions with our earlier publications on a smaller sample size in the EARTH cohort (Mok-Lin *et al.*, 2010; Ehrlich *et al.*, 2012b). First, Mok-Lin *et al.* (2010) reported that urinary BPA concentrations were inversely associated with the number of oocytes retrieved per cycle and peak serum estradiol levels in 84 women contributing to 112 IVF cycles (Mok-Lin *et al.*, 2010). Later, Ehrlich *et al.* (2012b) reported that women with higher urinary BPA concentrations had significantly lower serum peak E2, oocyte yield, MII oocyte count, and number of normally fertilizing oocytes among 174 women contributing 237 cycles (Ehrlich *et al.*, 2012b). Previously, we also published an analysis that found a suggestive relationship (P, trend = 0.12) between higher urinary BPA concentrations and increased implantation failure among 137 women undergoing 180 IVF cycles (Ehrlich *et al.*, 2012a). However, in our present analyses, we did not find an association between higher urinary BPA concentrations and lower implantation. (Note that in the current analysis we explored associations with implantation rather than implantation failure.) To better compare our current results on a larger sample size to our earlier published results, we ran several sensitivity analyses. The inverse association of urinary BPA with oocyte yield and serum peak E2, and the borderline association between BPA and implantation found previously were not confirmed in the present larger and more powerful analysis. We determined, by comparing our previous and current analyses, that time trends in urinary BPA concentrations or IVF success rates could not account for differences in results. Furthermore, although our earlier analysis was per embryo transfer whereas our current was per initiated cycle, we ran sensitivity analyses and determined that this did not account for difference in results. Therefore, the most likely explanation is that our earlier preliminary analysis on a much smaller sample size might have yielded spurious findings that differed from the current analysis with larger sample size. There are few other studies on BPA and reproductive outcomes among women from a fertility clinic. A small study conducted at the University of California in San Francisco, found no associations of serum BPA concentrations with embryo cleavage rate or fragmentation in 27 women undergoing IVF and participating in the prospective cohort Study of Metals and Assisted Reproductive Technologies (Bloom *et al.*, 2011b). Accordingly, the Longitudinal Investigation of Fertility and the Environmental Study showed that neither female nor male BPA urinary concentrations were associated with time to pregnancy among 501 couples recruited upon discontinuing contraception to become pregnant between 2005 and 2009 (Buck Louis *et al.*, 2014).

We also found no association between urinary BPA concentrations with clinical pregnancy and live birth in our study population. Due to scarce human data, we considered it important to compare our result with experimental animal data. Consistent with our results, BPA exposure was not associated with the number of live pups or total number of delivered pups (Howdeshell *et al.*, 2008; Tyl *et al.*, 2008; Thuillier *et al.*, 2009; Kobayashi *et al.*, 2010, 2012; Ryan *et al.*, 2010; Xi *et al.*, 2011; Nanjappa *et al.*, 2012) in mice and rats. However, other studies showed contradictory results in the same type of animals (Salian *et al.*, 2009a,b; Cabaton *et al.*, 2011). Despite these inconsistent results on pregnancy outcomes, there is experimental evidence for effects of BPA on folliculogenesis and oocyte meiosis. A recent review concluded that in animal models, BPA adversely affects oocyte meiosis, interferes with germ cell nest breakdown, reduces the primordial follicle pool by stimulating their initial recruitment and subsequent follicle development until the antral stage, alters ovarian steroidogenesis, modifies normal uterine morphology, and impairs uterine receptivity and ova-implantation (Peretz *et al.*, 2014).

Although we did not find any association between urinary BPA concentrations and IVF outcomes in this study population, the relation between BPA and endometrial wall thickness was modified by age. Younger women (<37 years old) had thicker endometrial thickness across increasing quartiles of urinary BPA concentrations while older women (≥ 37 years old) had thinner endometrial thickness across increasing quartiles of urinary BPA concentrations. It is unclear why this association was modified by age and further studies are needed to corroborate this finding.

Our study has several limitations worth noting. One limitation is the uncertainty of extrapolating our results to women from the general population. Also, misclassification of BPA exposure based on spot urine sample is possible because BPA is a short-lived chemical and exposures are likely to be episodic in nature (Ye *et al.*, 2011; Braun *et al.*, 2012; Lassen *et al.*, 2013). This would likely attenuate associations. Strengths of our study include its prospective design which minimizes the risk of reverse causation, the comprehensive adjustment of possible confounding variables, and the adequate power (80%) of the study which was able to detect clinically relevant difference of 29% in clinical pregnancy rates and 27% in live birth rates between women in the top and bottom quartiles of urinary BPA concentrations.

In conclusion, we found little evidence of adverse associations of urinary BPA concentrations with reproductive and pregnancy outcomes among women from a fertility clinic. We are currently using the same cohort to explore associations of reproductive and pregnancy outcomes with additional chemicals, such as phthalates and parabens. Furthermore, when there is an adequate sample size, we propose to explore potential effects of mixtures that include BPA on reproductive health.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

R.H. and J.E.C. were involved in study concept and design, and critical revision for important intellectual content of the manuscript. P.L.W. contributed to method modification and provided statistical expertise. L.M.-A. analyzed data, drafted the manuscript and had a primary responsibility for final content. L.M.-A., Y.-H.C., J.E.C., P.L.W. and R.H. interpreted the data. S.E. contributed to the statistical analyses. A.J.G. reviewed the statistical analysis. J.C.P., J.B.F. and A.M.C. were involved in acquisition of the data. All authors were involved in the critical revision of the manuscript and approved the final manuscript.

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Conflict of interest

None of the authors has any conflicts of interest to declare. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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