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Bisphenol A exposure reduces the estradiol response to gonadotropin stimulation during *in vitro* fertilization

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Abstract

Objective—Investigate associations between serum BPA concentrations and follicular response to exogenous ovary stimulation.

Design—Fasting serum was prospectively collected on the day of oocyte retrieval and assessed for unconjugated BPA using high-performance liquid chromatography (HPLC) with Coularray detection. Multivariable linear regression and negative binomial regression were used to assess associations between concentrations of BPA and outcome measures. Models were adjusted for race/ethnicity, antral follicle count at baseline, and cigarette smoking.

Setting—A reproductive health center.

Patients—Forty-four women undergoing *in vitro* fertilization (IVF).

Main outcome measures—Peak-estradiol level (E_2) and the number of oocytes retrieved during IVF.

Results—The median unconjugated serum BPA concentration is 2.53 ng/ml (range 0.3–67.36 ng/ml). Bisphenol A is inversely associated with E_2 (β=−0.16; 95% confidence interval (CI) −0.32, 0.01), as well as with E_2 normalized to the number of mature-sized follicles at the hCG trigger (β=−0.14; 95%CI −0.24, −0.03). No association is observed for BPA and the number of oocytes retrieved (adjusted risk ratio=0.95; 95%CI 0.82, 1.10).

Conclusions—Bisphenol A is associated with a reduced estradiol response during IVF. Although limited by the preliminary nature of this study, these results merit confirmation in a future comprehensive investigation.

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Keywords

Bisphenol A (BPA); Estradiol (E_2); Follicle; *In vitro* fertilization (IVF); Ovary

Introduction

Bisphenol A (BPA) is an endocrine-disrupting synthetic compound used widely in commercial products, canned food containers, water pipes, and dental sealants (1). Although it has a short ½-life (< 6 h) *in vivo* (2), BPA has been detected in concentrations exceeding 0.1 μg/L in over 90% of urine samples collected from U.S. residents (3). Studies have also reported BPA in blood, follicular fluid (4, 5), semen (5, 6), and amniotic fluid (7). Other studies have indicated estrogenic properties for BPA *in vitro* (8), and *in vivo* using murine models (9). Moreover, disrupted oocyte meiosis has been demonstrated in association with BPA exposure (8, 10). In addition, *in vitro* studies have shown mitotic spindle defects and meiotic arrest following BPA exposure during oocyte maturation (11–13). BPA also inhibits estradiol production in granulosa cells (14–17).

We recently reported an inverse association between serum unconjugated BPA concentrations and oocyte fertilization among infertile couples undergoing *in vitro* fertilization (IVF) (18). Serial estradiol measurements are used clinically as surrogate markers for follicular response and health during IVF. Total urine BPA concentrations have been negatively correlated with both peak serum estradiol (E_2) levels and the number of oocytes retrieved in women undergoing IVF (19). Despite these results, the dearth of published research on BPA toxicity constitutes a critical gap in our understanding of environmental influences on human reproduction. In this preliminary study, we extend our previous study of BPA and oocyte quality (18) with an assessment of associations between serum BPA, peak-E_2 concentrations, and the number of oocytes retrieved during IVF in the same study population.

Materials and Methods

The study cohort consists of 58 female patients and 37 male partners completing a 1st IVF cycle at the University of California at San Francisco (UCSF) Center for Reproductive Health between 3/12/07 and 4/29/08. Forty-four women with data available for serum unconjugated BPA concentration, and with peak-E_2 level or the number of oocytes retrieved are included in this study. None had previously completed a cycle of IVF, but n=3 had previously initiated a single cancelled IVF cycle; to maximize the limited sample size these women were retained in the analysis. There are no differences between women with and without BPA concentrations in terms of age, cigarette smoking, race/ethnicity or body mass index (BMI) (data not shown). Informed consent was obtained and the study protocol was approved by the UCSF Committee for Human Research.

The clinical protocol and sample collection have been previously described (20). Basal follicle stimulating hormone (FSH) concentrations were abstracted from medical records. Antral follicle count (AFC) was assessed by transvaginal ultrasonography (TVU) during initial consultation. Women underwent gonadotropin-induced ovarian stimulation according to clinic protocols. Follicle maturation was routinely assessed by TVU and E_2 levels throughout the stimulation. Estradiol concentrations were measured by a solid-phase competitive chemiluminescent enzyme immunoassay using an Immulite 1000 analyzer (Siemens Healthcare Diagnostics, Deerfield, IL). Human chorionic gonadotropin (5000–10,000 IU hCG) was administered subcutaneously after at least two follicles of 16–18 mm diameter were observed, and oocytes were retrieved 36 hours later. Prior to the hCG trigger

the total number of follicles ≥13 mm diameter ('mature-sized follicles') was recorded. A blood specimen was obtained from women at the time of oocyte retrieval; women were asked to fast for a minimum of eight hours prior to the procedure.

Blood specimens were collected into serum separator Vacutainer® tubes (Becton Dickinson and Co., Franklin Lakes, NJ), let sit for 20–30 minutes, and centrifuged at 700 g for 10 minutes. Serum was aliquoted into 1.8 mL polypropylene cryovials and immediately frozen at −80 ºC. Serum specimens were shipped on dry ice to the Endocrine Disruptors Laboratory at the University of Missouri (Columbia, MO) in September, 2008. Concentrations of unconjugated BPA were determined in serum by high performance liquid chromatography (HPLC) with an ESA Coularray 5600 detector (ESA Inc., Chelmsford, MA) as described in detail elsewhere (18). Using an internal BPA standard, separate and distinct chromatographic peaks were captured (Supplemental Figure 1). Recoveries were estimated by comparing BPA concentrations in human serum samples before and after spiking with 5 ng BPA/mL (MP Biomedicals, Solon, OH USA); averaging 89%. For values measured below the lowest on column standard (i.e., 0.05 ng), we extrapolated from the standard curve and used these extrapolated values in the statistical analysis. The limit of detection (LOD) under these conditions was 0.3 ng/ml serum. For samples with no evidence for the presence of BPA at any concentrations, we assigned a value of zero. Empty serum collection tubes and laboratory diluent and extraction blanks did not contain detectable BPA. The concentration of BPA in serum was unrelated to the volume of serum measured (R^2=0.002), which varied from 0.5 – 2.0 mL (mean serum volume extracted=1.45 mL). To preclude the introduction of bias demonstrated when censoring values below LODs (21), machine-read values were reported for samples below the LOD.

Demographics, serum BPA measures and clinical measures were characterized by descriptive statistics. A natural log transformation was applied to serum BPA concentration (following the addition of 1.0 to accommodate zeros), peak-E_2, body mass index (BMI), and baseline AFC to provide normal distributions. Pearson correlation coefficients and ANOVA were employed to evluate associations between BPA concentrations and continous and categorical variables, respectively, including age, dichotomized race/ethnicity ("not-Asian" vs. "Asian"), cigarette smoking ("never" vs. "ever"), infertility diagnosis, peak-E_2 concentration, baseline AFC, basal FSH concentration, and the number of oocytes retrieved. Statistical significance was defined as $P<0.05$ for a two-tailed test. Statistical analysis was performed using SAS v.9.2 (SAS Institute, Cary, NC).

Multivariable linear regression was used to estimate the association of BPA concentration with peak-E_2 while adjusting for selected covariates in 42 women with complete covariate data (excluded n=1 missing peak-E_2 and n=1 missing AFC). This model was repeated after normalizing peak-E_2 concentration to the number of mature-sized follicles at the time of the hCG trigger (22). Residual analysis was conducted using scatter plots and regression diagnostics to identify potentially influential observations for further consideration (23). To accommodate over dispersion in the dependent variate, multivariable negative binomial regression was used to evaluate associations between BPA concentration and the number of oocytes retrieved, in 43 women with complete covariate data (excluded n=1 missing AFC). Model coefficients and their 95% confidence intervals were exponentiated to provide risk ratios. Patient race/ethnicity (3), cigarette smoking (24), and ovarian reserve (25) were *a priori* selected as confounding variables for inclusion with serum BPA concentrations in multivariable regression models, based on literature review and incorporation into directed acyclic graphs (DAGs) (26). We considered chronologic age, basal FSH, and AFC as markers for ovarian reserve (27); AFC was ultimately selected as it provided the best model fit. The coefficient of determination (R^2), log-likelihood values, and deviance statistics were used to assess goodness-of-fit for multivariable linear regression, and for multivariable

negative binomial regression models as appropriate (23). We did not adjust for prior IVF cycle cancellation despite reported associations between the outcomes of IVF cycles (28). We do not anticipate current relevance for historical BPA exposure, that which may have predicted cancellation in a prior cycle; the ½-life of BPA is measured in hours (2). It is inappropriate to adjust for prior reproductive outcomes in this context (29).

Results

Descriptors for the study sample are provided in Table 1. At the time of oocyte retrieval, women were a mean (±SD) 35.8 (±4.1) years of age. Most were never-smokers (84.1%) and a substantial proportion Asian (29.6%). Median serum BPA concentration was 2.5 ng/ml (range=0.0, 67.4 ng/ml); a majority exceeded the LOD (86.4%). Peak-E_2 reached a median of 2167.0 pg/mL serum during ovarian stimulation, and a median of 10.5 oocytes (range=2, 35) were retrieved from each woman. Prior to ovarian stimulation, a median of 10.0 baseline antral follicles (range=2, 29) were counted for each woman, and just prior to the hCG trigger a median 9.0 mature-sized follicles (range=2, 25) were visualized. Basal FSH was a median 6.4 IU/L serum (range=0.0, 12.5). Approximately 36.4% of the infertility diagnoses were unexplained infertility, while 20.5% carried the diagnosis with diminished ovarian reserve, and an additional 20.5% were male infertility diagnoses.

Bivariate associations between BPA, peak-E_2 and demographic and clinical factors are summarized in Table 2. Women reporting a race/ethnicity not-Asian demonstrate higher median serum BPA concentrations (2.85 ng/ml) than Asian women (1.24 ng/ml); albeit of 'borderline' statistical significance (P=0.10). Serum BPA does not vary by cigarette smoking (P=0.40), or by primary infertility diagnosis (P=0.19), nor does it correlate with age (r=−0.05, P=0.77). However, inverse correlations are suggested for BPA with peak-E_2 (r=−0.29, P=0.06), and for BPA with peak-E_2 per mature-sized follicle (r=−0.33, P=0.03). In contrast, no associations are indicated for BPA with baseline AFC (r=−0.09, P=0.55), with basal FSH (r=0.23, P=0.13) or with number of oocytes retrieved (r=−0.13, P=0.40). Not surprisingly, peak-E_2 demonstrates correlations with the number of mature-sized follicles (r=0.86, P<0.0001), baseline AFC (r=0.60, P<0.0001), the number of oocytes retrieved (r=0.70, P<0.0001), and an association is suggested for basal FSH concentrations (r=−0.27, P=0.08), although not for age (r=−0.08 P=0.62). Exclusion of n=3 with a diagnosis of 'anovulation' has little impact on the magnitude of the peak-E_2-AFC correlation (r=0.63, P<0.0001). Median concentrations of peak-E_2 and peak-E_2 per mature-sized follicle are higher among never-smokers (2584.5 pg/mL serum and 277.2, respectively) than among ever-smokers (1311.0 pg/mL serum and 231.4, respectively), albeit of borderline statistical significance (P=0.09 and 0.10, respectively). The number of oocytes retrieved is correlated to number of mature-sized follicles (r=0.78, P<0.0001), with a trend for FSH concentrations (r=−0.26, P=0.08); however, not with age (r=0.04, P=0.77) or cigarette smoking (P=0.19).

Multivariable linear regression models suggest an inverse association between serum BPA concentration and peak-E_2, adjusted for race/ethnicity, cigarette smoking and baseline AFC (Table 3, Supplemental Figure 2). In one model, a log-unit increase in BPA is associated with a decrease of −0.16 units in log peak-E_2 (β=−0.16, 95%CI=−0.32, 0.01; P=0.07), although of borderline statistical significance. Removal of confounding variables from the model reduces the magnitude of the BPA coefficient by 13.5% (data not shown). In a second model, the same increase in serum BPA is associated with a statistically significant decrease of −0.14 units in log peak-E_2 per mature-sized follicle (β=−0.14, 95%CI=−0.24, −0.03; P=0.01). Removal of confounding variables from the model reduces the magnitude of the BPA coefficient by 28.6% (data not shown). In contrast, no association is indicated for serum BPA concentrations and the number oocytes retrieved as demonstrated in Table 4

(adjusted risk ratio (aRR)=0.95, 95%CI=0.82, 1.10; P=0.49). Removal of confounding variables from the model reduces the magnitude of the BPA coefficient by 55.1% (data not shown). Removal of only AFC as a covariate from the model marginally increases the magnitude and precision of the BPA effect estimate (aRR=0.91, 95%CI=0.77–1.08; P=0.28).

Discussion

In this preliminary cohort study, we describe inverse associations between unconjugated serum BPA concentrations and peak-E_2 levels in women undergoing IVF. This result is underscored by similar and more precise results generated by a model in which peak-E_2 is normalized to the number of mature-sized follicles at the time of the hCG trigger (i.e., follicles ≥13 mm diameter). These associations were adjusted for race/ethnicity, cigarette smoking, and AFC. We did not find any associations between BPA concentrations and ovarian reserve variables such as AFC and basal FSH concentrations. Furthermore, we did not observe evidence for an association between BPA and the number of oocytes retrieved in crude or adjusted analyses.

Similar results were recently reported by Mok-Lin *et al*. (19), in a study of urine total BPA concentrations and peak serum E_2 levels conducted in a sample of 84 women undergoing IVF. The authors reported an average decrease of 213 pg/mL (95%CI=−407, −20) in peak-E_2 per log unit increase in urine total BPA. We detect average adjusted decreases of 106 pg/mL (95%CI=−201, 8), and 50 pg/mL (95%CI=−83, −13) in peak-E_2, and peak-E_2 per mature-sized follicle in the current study, respectively for each log unit increase in serum unconjugated BPA. These results are substantiated by an increasing body of *in vitro* literature which reports reduced granulosa cell estrogen synthesis in association with BPA treatment (14–17). Evidence from studies *in vitro* suggests these reductions are secondary to inhibition of FSH stimulated granulosa cell aromatase activity (15, 17, 30–32) required for theca cell conversion of androgens to estrogens (33). Additional evidence indicates a role for the peroxisome proliferators-activated receptor γ(PPAR γ), which inhibits aromatase activity in response to BPA exposure (15).

Several investigators report inhibition of oocyte development following BPA treatment *in vitro* (10–13), and thus we also anticipated an inverse association between BPA exposure and oocytes retrieved *a priori*. Although not statistically significant as are the results of the recent study by Mok-Lin *et al*., (19), which reports an average 12% decrease (95%CI= −23%, −4%) in the number of oocytes retrieved per log unit increase in urine total BPA, our data are consistent with a subtle inverse association, and our lower 95% confidence limit is consistent with those reported results. Exclusion of AFC as a covariate from our model, considering the possibility that this variable may fall within the causal pathway, increased the magnitude of the effect estimate to −9% with a confidence interval similar to, but wider than that previously reported (−23%, 8%). Our ability to detect an association is limited by our sample size, which is a fraction of the 112 cycles evaluated by Mok-Lin *et al*.

The difference in results between our study and that reported by Mok-Lin and colleagues (19) might be attributable in part to differential rates of misclassification of the oocytes retrieved endpoint. Oocyte retrieval is vulnerable to misclassification bias as human operator error may result in ‘empty follicles’, *vis a vis* inadvertent loss of retrieved oocytes (34). However, the average number of oocytes reported previously (mean±SD=10.4±5.3) is similar to the current study and thus oocyte retrieval error is an unlikely explanation for the discrepancy. An alternate explanation pertains to the use of distinctly different exposure assessment strategies. Total urine BPA, measured in the former study (19), is comprised of both conjugated and unconjugated fractions, but dominated by the biologically-inactive

conjugated form (2). Serum unconjugated BPA, measured among participants in the current study, is comprised only of that fraction which is biologically-active (35). Unfortunately, differences in biologic matrices and BPA fractions considered preclude a comparison of exposure levels between the two studies. However, metabolic factors not captured by our approach might play a role in the association reported Mok-Lin and colleagues. A third possible explanation for the discrepant results between the two studies pertains to differences in confounder adjustment strategies. However, replication of the oocyte retrieval model reported by Mok-Lin *et al* does not substantially alter our results (aRR=0.97, 95%CI=0.89, 1.05; P=0.43). A future study, in which both urine total BPA and serum unconjugated BPA are measured in women undergoing IVF will be necessary to resolve the discrepant oocyte retrieval results between our study and those reported previously.

The primary limitation of this study is the small size of the sample; we may be unable to detect small or even moderate associations, or to make firm conclusions regarding associations between serum BPA concentrations, peak-E_2 and the number of oocytes retrieved during IVF. In addition, we are unable to consider other clinical factors which might be statistically relevant. Factors such as infertility diagnosis or clinical protocol might modify associations between BPA exposure and ovarian response. However, the results of this study are corroborated by our detection of *a priori* anticipated bivariate associations between peak-E_2 concentrations and the number of mature-sized follicles at the hCG trigger, baseline AFC, basal FSH concentrations, and the number of oocytes retrieved (25, 27, 36). Race/ethnicity does not appear to be associated with ovarian response, peak-E_2 or with the number of oocytes retrieved, although higher peak-E_2 levels were recently reported for Asian women in this study population (22, 37); this discrepancy is likely a result of the limited number of participants in this study. Lower E_2 concentrations are suggested for ever-smokers, compared to never-smokers as expected *a priori* (38), and the average number of oocytes retrieved among never-smokers exceeds that of ever-smokers; however, this difference does not approach statistical significance.

The results of this study are strengthened by our assessment of circulating unconjugated BPA, which provides a more direct assessment of internal dose when compared with prior reports considering urine concentrations of total, conjugated or unconjugated BPA (39). Dietary sources are purported to provide the overwhelming majority of human BPA exposure (40); however, the concentrations of unconjugated BPA detected in our study are consistent with growing evidence eroding this presumption, and that there are additional as of yet unidentified sources of non-dietary exposure (41–43). Women were asked to fast prior to oocyte collection; this is confirmed on the morning of oocyte retrieval. However, there may still be variability between women in the time since last meal and this may introduce measurement error into our exposure assessment. We do not anticipate an association between time since last meal and peak-E_2, or with the number of oocytes retrieved. Bias associated with fasting variability is therefore likely to be towards the null hypothesis.

Another strength of our study is the use of a Coularray detection method following HPLC separation for the measurement of serum unconjugated BPA. In terms of sensitivity and specificity, this approach is considered superior to the less resource intensive and more frequently employed ELISA approach for the measurement of BPA in human serum (44). Moreover, the possibility for spontaneous deconjugation of glucuronidated BPA to the unconjugated form is not likely in our study given the storage of specimens at −80º C for limited duration (i.e., 3–19 months), and the known stability of conjugated BPA during extraction with organic solvents (45). The use of polycarbonate products for the collection, processing and storage of serum specimens has raised concern regarding possible BPA contamination of specimens (46). No polycarbonate or epoxy resin containing supplies were employed, to our knowledge, during specimen collection, processing, and storage; however,

this issue remains a possibility. Still, our dataset is limited by the absence of follicular fluid measures, which are presumed to provide a more direct and relevant assessment of BPA dose as well as E_2 level.

Consistent with the clinical IVF protocol, we captured serum E_2 measures across the cycle and were thereby able to assess peak-E_2 as a study endpoint. These data were gathered using an automated chemiluminescent immunoassay, rather than by use of a reference method such as gas chromatography with mass spectrometry, raising the specter of cross-reactivity (47). Previous report indicates minimal if any cross-reactivity with other estrogenic compounds and thus we do not anticipate interference with E_2 measures by BPA (48). However, we acknowledge this possibility and suggest that if an interference is present, a bias towards a positive association between serum BPA and peak-E_2 would result, opposite to the effect we report herein.

We provide preliminary evidence suggesting that BPA exposure may influence the E_2 response during gonadotropin stimulation, with and without normalization to the number of mature–sized follicles seen. In contrast, our preliminary findings do not find any associations between BPA exposure and ovarian reserve parameters including AFC, basal FSH, and the number of oocytes retrieved. Our results suggest that BPA may explain in part the heterogeneity of E_2 responses to gonadotropin stimulations seen in a clinic IVF population. Additional studies are needed to confirm the effect of BPA on E_2 response and to assess the biological basis for the effect. Should this association be confirmed clinicians might advise patients to avoid known sources of exposure to BPA sources (49), in an effort to reduce the variability of E_2 responses to gonadotropin stimulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Description of women undergoing *in vitro* fertilization, and participating in the Study of Metals and Assisted Reproductive Technologies (SMART).

Variables	n	(%)	Mean	SD	Min	25th%tile	Median	75th%tile	Max
Age (Years)	44	-	35.80	4.08	28.00	32.00	36.00	38.00	44.00
BMI (kg/m²)	41	-	24.28	4.90	17.85	21.16	22.99	25.91	45.58
Smoking									
Never	37	84.09	-	-	-	-	-	-	-
Ever	7	15.91	-	-	-	-	-	-	-
Race/ethnicity									
Not Asian	31	70.45	-	-	-	-	-	-	-
Asian	13	29.55	-	-	-	-	-	-	-
BPA (ng/mL serum)	44	-	7.22	14.15	0.00	0.52	2.53	6.31	67.36
Above LOD (0.3 ng/mL serum)	38	86.36	-	-	-	-	-	-	-
Peak-E_2 (pg/ml serum)	43	-	2736.35	1732.59	383.00	1305.00	2167.00	3707.00	8456.00
Number of oocytes retrieved	44	-	12.07	7.36	2.00	6.00	10.50	16.50	35.00
Baseline AFC	43	-	12.00	6.97	2.00	6.00	10.00	17.00	29.00
Mature-sized (≥13 mm) follicles at hCG trigger	44	-	10.05	5.63	2.00	5.50	9.00	15.00	25.00
FSH (IU/L)	44	-	6.65	2.91	0.00	5.05	6.40	8.11	12.50

AFC, antral follicle count; BMI, body mass index; BPA, bisphenol A; FSH, follicle stimulating hormone; hCG, human chorionic gonadotropin; LOD, limit of detection; Max,. maximum value; Min., minimum value; SD, standard deviation.

Table 2

Bivariate associations between serum BPA, and demographic and clinical factors for women undergoing *in vitro* fertilization, and participating in the Study of Metals and Assisted Reproductive Technologies (SMART).

Factors	n	Correlation coefficient or F-value	P-value
BPA [a] with:			
Asian race/ethnicity	44	2.86	0.10
Ever smoking	43	0.71	0.40
Infertility diagnosis	44	1.55	0.19
Age	44	−0.05	0.77
BMI [a, b]	41	−0.12	0.44
Peak-E_2 [a, c]	43	−0.29	0.06
# Mature-sized follicles (≥13 mm)	44	−0.10	0.50
Peak-E_2 per follicle ≥13 mm [a, c]	43	−0.33	0.03
AFC [a, d]	43	−0.09	0.55
FSH	44	0.23	0.13
# Oocytes retrieved	44	−0.13	0.40
Peak-E_2 [a, c] with:			
Asian race/ethnicity	43	0.86	0.36
Ever smoking	43	2.94	0.09
Infertility diagnosis	43	1.19	0.01
Age	43	−0.08	0.62
BMI [a, b]	40	−0.23	0.16
# Mature-sized follicles (≥13 mm)	43	0.86	<0.0001
AFC [a, d, e]	42	0.60	<0.0001
FSH	43	−0.27	0.08
# Oocytes retrieved	43	0.70	<0.0001
# Ooctyes retrieved with:			
Asian race/ethnicity	44	0.58	0.45
Ever smoking	44	1.76	0.19
Infertility diagnosis	44	2.57	0.04
Age	44	0.04	0.77
BMI [a, b]	41	0.00	0.98
# Mature-sized follicles (≥13 mm)	44	0.78	<0.0001
AFC [a, d, e]	43	0.54	0.0002
FSH	44	−0.26	0.08

[a] Natural log transformed;

[b] n=3 missing values for BMI;

[c] n=1 missing value for peak-E_2;

[d] n=1 missing value for AFC;

[e] excluding n=3 with anovulation; r=0.63, P<0.0001.

AFC, antral follicle count; BMI, body mass index; BPA, bisphenol A; CI, confidence interval; E_2, estradiol; FSH, follicle stimulating hormone.

Table 3

Multivariable linear regression models for associations between serum unconjugated BPA concentration and peak serum E_2 in 42 women undergoing *in vitro* fertilization, and participating in the Study of Metals and Assisted Reproductive Technologies (SMART).

Predictors	Model for peak-E_2 [a,b]				Model for peak-E_2 per mature-sized follicle [a-c]			
	Beta	Low 95%CI	High 95%CI	P-value	Beta	Low 95%CI	High 95%CI	P-value
BPA	−0.16	−0.32	0.01	0.07	−0.14	−0.24	−0.03	0.01
Race/Ethnicity	−0.05	−0.46	0.36	0.82	−0.11	−0.37	0.14	0.36
Smoking	−0.40	−0.90	0.10	0.12	−0.34	−0.65	−0.03	0.03
Baseline AFC	0.60	0.32	0.88	0.0001	−0.07	−0.24	0.11	0.45

[a] Natural log transformed.

[b] n=1 with missing peak-E_2 excluded, and n=1 with missing AFC excluded;

[c] Peak-E_2 divided by number of ovarian follicles ≥13 mm diameter at the time of the hCG trigger.

AFC, antral follicle count; BPA, bisphenol A; CI, confidence interval; E_2, estradiol; hCG, human chorionic gonadotropin.

Table 4

Multivariable negative binomial regression for the association between serum unconjugated BPA concentration and the number of oocytes retrieved in 43 women undergoing *in vitro* fertilization, and participating in the Study of Metals and Assisted Reproductive Technologies (SMART).

Predictor	aRR	Low 95%CI	High 95%CI	P-value
BPA [a]	0.95	0.82	1.10	0.49
Race/Ethnicity	1.06	0.75	1.49	0.75
Smoking	0.79	0.51	1.24	0.31
Baseline AFC [a, b]	1.77	1.38	2.28	<0.0001

[a] Natural log transformed;

[b] n=1 with missing AFC excluded.

AFC, antral follicle count; aRR, adjusted risk ratio; BPA, bisphenol A; CI, confidence interval.