

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

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**Objective:** To investigate associations between serum bisphenol A (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 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.

**Patient(s):** Forty-four women undergoing IVF.

**Intervention(s):** None.

**Main Outcome Measure(s):** Peak E<sub>2</sub> level and the number of oocytes retrieved during IVF.

**Result(s):** 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<sub>2</sub> ( $\beta = -0.16$ ; 95% confidence interval =  $-0.32, 0.01$ ), as well as with E<sub>2</sub> normalized to the number of mature-sized follicles at the hCG trigger ( $\beta = -0.14$ ; 95% confidence interval =  $-0.24, -0.03$ ). No association is observed for BPA and the number of oocytes retrieved (adjusted risk ratio = 0.95; 95% confidence interval = 0.82, 1.10).

**Conclusion(s):** Bisphenol A is associated with a reduced E<sub>2</sub> response during IVF. Although limited by the preliminary nature of this study, these results merit confirmation in a future comprehensive investigation. (*Fertil Steril*® 2011;96:672–7. ©2011 by American Society for Reproductive Medicine.)

**Key Words:** Bisphenol A (BPA), estradiol (E<sub>2</sub>), follicle, in vitro fertilization (IVF), ovary

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 half-life (<6 h) in vivo (2), BPA has been detected in concentrations exceeding 0.1  $\mu\text{g/L}$  in more than 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 E<sub>2</sub> production in granulosa cells (14–17).

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We recently reported an inverse association between serum unconjugated BPA concentrations and oocyte fertilization among infertile couples undergoing IVF (18). Serial E<sub>2</sub> 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 E<sub>2</sub> 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<sub>2</sub> 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 first IVF cycle at the University of California at San Francisco Center for Reproductive Health between March 12, 2007 and April 29, 2008. Forty-four women with data available for serum unconjugated BPA concentration, and with peak E<sub>2</sub> level or the number of oocytes retrieved are included in this study. None had previously completed a cycle of IVF, but three 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 (data not shown). Informed consent was obtained and the study protocol was approved by the University of California at San Francisco Committee for Human Research.

The clinical protocol and sample collection have been previously described (20). Basal FSH concentrations were abstracted from medical records. Antral follicle count (AFC) was assessed by using transvaginal ultrasonography during initial consultation. Women underwent gonadotropin-induced ovarian stimulation according to clinic protocols. Follicle maturation was routinely assessed by transvaginal ultrasonography and E<sub>2</sub> levels throughout the stimulation. Estradiol concentrations were measured by a solid-phase competitive chemiluminescent enzyme immunoassay using an Immulite 1000 analyzer (Siemens Healthcare Diagnostics). 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. Before 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 8 hours before the procedure.

Blood specimens were collected into serum separator Vacutainer tubes (Becton Dickinson and Co.), 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°C. Serum specimens were shipped on dry ice to the Endocrine Disruptors Laboratory at the University of Missouri (Columbia) in September 2008. Concentrations of unconjugated BPA were determined in serum by using high-performance liquid chromatography with an ESA Couarray 5600 detector (ESA Inc.) as described in detail elsewhere (18). Using an internal BPA standard, separate and distinct chromatographic peaks were captured (Supplemental Fig. 1). Recoveries were estimated by comparing BPA concentrations in human serum samples before and after spiking with 5 ng BPA/mL (MP Biomedicals), 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 to 2.0 mL (mean serum volume extracted = 1.45 mL). To preclude the introduction of bias demon-

strated when censoring values below limits of detection (21), machine-read values were reported for samples below the limit of detection.

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<sub>2</sub>, body mass index, and baseline AFC to provide normal distributions. Pearson correlation coefficients and ANOVA were employed to evaluate associations between BPA concentrations and continuous and categorical variables, respectively, including age, dichotomized race/ethnicity (“not-Asian” vs “Asian”), cigarette smoking (“never” vs “ever”), infertility diagnosis, peak E<sub>2</sub> concentration, baseline AFC, basal FSH concentration, and the number of oocytes retrieved. Statistical significance was defined as  $P < .05$  for a two-tailed test. Statistical analysis was performed using SAS v.9.2 (SAS Institute).

Multivariable linear regression was used to estimate the association of BPA concentration with peak E<sub>2</sub> while adjusting for selected covariates in 42 women with complete covariate data (excluding  $n = 1$  missing peak E<sub>2</sub> and  $n = 1$  missing AFC). This model was repeated after normalizing peak E<sub>2</sub> 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 overdispersion 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 (excluding  $n = 1$  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, on the basis of a literature review and incorporation into directed acyclic graphs (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 half-life of BPA is measured in

**TABLE 1**

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

Variables	n	%	Mean	SD	Min.	25%	Median	75%	Max.
Age (y)	44	–	35.80	4.08	28.00	32.00	36.00	38.00	44.00
BMI	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 <sub>2</sub> (pg/mL serum)	43	–	2736.35	1732.59	383.00	1305.00	2167.00	3707.00	8456.00
No. 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

Note: BMI = body mass index; LOD = limit of detection; Max = maximum value; Min = minimum value.

Bloom. Serum bisphenol A and estradiol response. *Fertil Steril* 2011.

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 ( $\pm$ SD) 35.8 ( $\pm$ 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 limit of detection (86.4%). Peak E<sub>2</sub> 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. Before ovarian stimulation, a median of 10.0 baseline antral follicles (range = 2–29) were counted for each woman, and just before 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, whereas 20.5% carried the diagnosis with diminished ovarian reserve, and an additional 20.5% were male infertility diagnoses.

Bivariate associations between BPA, peak E<sub>2</sub> level, 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 ( $P=.10$ ). Serum BPA does not vary by cigarette smoking ( $P=.40$ ), or by primary infertility diagnosis ( $P=.19$ ), nor does it correlate with age ( $r = -.05$ ,  $P=.77$ ). However, inverse correlations are suggested for BPA with peak E<sub>2</sub> ( $r = -.29$ ,  $P=.06$ ), and for BPA with peak E<sub>2</sub> per mature-sized follicle ( $r = -.33$ ,  $P=.03$ ). In contrast, no associations are indicated for BPA with baseline AFC ( $r = -.09$ ,  $P=.55$ ), with basal FSH ( $r = .23$ ,  $P=.13$ ) or with number of oocytes retrieved ( $r = -.13$ ,  $P=.40$ ). Not surprisingly, peak E<sub>2</sub> demonstrates correlations with the number of mature-sized follicles ( $r = .86$ ,  $P<.0001$ ), baseline AFC ( $r = .60$ ,  $P<.0001$ ), the number of oocytes retrieved ( $r = .70$ ,  $P<.0001$ ), and an association is suggested for basal FSH concentrations ( $r = -.27$ ,  $P=.08$ ), although not for age ( $r = -.08$ ,  $P=.62$ ). Exclusion of  $n = 3$  with a diagnosis of anovulation has little impact on the magnitude of the peak E<sub>2</sub>–AFC correlation ( $r = .63$ ,  $P<.0001$ ). Median concentrations of peak E<sub>2</sub> and peak E<sub>2</sub> per mature-sized follicle are higher among never-smokers (2584.5 and 277.2 pg/mL serum, respectively) than among ever-smokers (1311.0 and 231.4 pg/mL serum, respectively), albeit of borderline statistical significance ( $P=.09$  and  $.10$ , respectively). The number of oocytes retrieved is correlated to number of mature-sized follicles ( $r = .78$ ,  $P<.0001$ ), with a trend for FSH concentrations ( $r = -.26$ ,  $P=.08$ ), although not with age ( $r = .04$ ,  $P=.77$ ) or cigarette smoking ( $P=.19$ ).

Multivariable linear regression models suggest an inverse association between serum BPA concentration and peak E<sub>2</sub>, adjusted for race/ethnicity, cigarette smoking, and baseline AFC (Table 3, Supplemental Fig. 2). In one model, a log unit increase in BPA is associated with a decrease of  $-0.16$  units in log peak E<sub>2</sub> ( $\beta = -0.16$ , 95% CI =  $-0.32$ ,  $0.01$ ;  $P=.07$ ), 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<sub>2</sub> per mature-sized follicle ( $\beta = -0.14$ , 95% CI =  $-0.24$ ,  $-0.03$ ;  $P=.01$ ). 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;  $P=.49$ ). Removal of confounding variables from the model reduces the magnitude of the BPA coefficient

**TABLE 2**

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

Factors	n	Correlation coefficient or <i>F</i> value	<i>P</i> value
<b>BPA<sup>a</sup> with</b>			
Asian race/ethnicity	44	2.86	.10
Ever smoking	43	0.71	.40
Infertility diagnosis	44	1.55	.19
Age	44	−0.05	.77
BMI <sup>a,b</sup>	41	−0.12	.44
Peak E <sub>2</sub> <sup>a,c</sup>	43	−0.29	.06
No. of mature-sized follicles ( $\geq 13$ mm)	44	−0.10	.50
Peak E <sub>2</sub> per follicle $\geq 13$ mm <sup>a,c</sup>	43	−0.33	.03
AFC <sup>a,d</sup>	43	−0.09	.55
FSH	44	0.23	.13
No. of oocytes retrieved	44	−0.13	.40
<b>Peak E<sub>2</sub><sup>a,c</sup> with</b>			
Asian race/ethnicity	43	0.86	.36
Ever smoking	43	2.94	.09
Infertility diagnosis	43	1.19	.01
Age	43	−0.08	.62
BMI <sup>a,b</sup>	40	−0.23	.16
No. of mature-sized follicles ( $\geq 13$ mm)	43	0.86	<.0001
AFC <sup>a,d,e</sup>	42	0.60	<.0001
FSH	43	−0.27	.08
No. of oocytes retrieved	43	0.70	<.0001
<b>No. of oocytes retrieved with</b>			
Asian race/ethnicity	44	0.58	.45
Ever smoking	44	1.76	.19
Infertility diagnosis	44	2.57	.04
Age	44	0.04	.77
BMI <sup>a,b</sup>	41	0.00	.98
No. of mature-sized follicles ( $\geq 13$ mm)	44	0.78	<.0001
AFC <sup>a,d,e</sup>	43	0.54	.0002
FSH	44	−0.26	.08

Note: BMI = body mass index; CI = confidence interval.

<sup>a</sup> Natural log transformed.

<sup>b</sup>  $n = 3$  missing values for BMI.

<sup>c</sup>  $n = 1$  missing value for peak E<sub>2</sub>.

<sup>d</sup>  $n = 1$  missing value for AFC.

<sup>e</sup> Excluding  $n = 3$  with anovulation;  $r = .63$ ,  $P<.0001$ .

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cient 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;  $P=.28$ ).

## DISCUSSION

In this preliminary cohort study, we describe inverse associations between unconjugated serum BPA concentrations and peak E<sub>2</sub> levels in women undergoing IVF. This result is underscored by similar and more precise results generated by a model in which peak E<sub>2</sub> is normalized to the number of mature-sized follicles at the time of the

**TABLE 3**

**Multivariable linear regression models for associations between serum unconjugated BPA concentration and peak serum E<sub>2</sub> in 42 women undergoing IVF, and participating in the Study of Metals and Assisted Reproductive Technologies (SMART).**

Predictors	Model for peak E <sub>2</sub> <sup>a,b</sup>				Model for peak E <sub>2</sub> per mature-sized follicle <sup>a-c</sup>			
	β	Low 95% CI	High 95% CI	P value	β	Low 95% CI	High 95% CI	P value
BPA <sup>a</sup>	-0.16	-0.32	0.01	.07	-0.14	-0.24	-0.03	.01
Race/ethnicity	-0.05	-0.46	0.36	.82	-0.11	-0.37	0.14	.36
Smoking	-0.40	-0.90	0.10	.12	-0.34	-0.65	-0.03	.03
Baseline AFC <sup>a</sup>	0.60	0.32	0.88	.0001	-0.07	-0.24	0.11	.45

Note: CI = confidence interval.  
<sup>a</sup> Natural log transformed.  
<sup>b</sup> n = 1 with missing peak E<sub>2</sub> excluded, and n = 1 with missing AFC excluded.  
<sup>c</sup> Peak E<sub>2</sub> divided by number of ovarian follicles ≥ 13 mm diameter at the time of the hCG trigger.

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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. Further, 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> and peak E<sub>2</sub> 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 that reports reduced granulosa cell estrogen synthesis in association with BPA treatment (14–17). Evidence from studies in vitro suggests that 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, which inhibits aromatase activity in response to BPA exposure (15).

**TABLE 4**

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

Predictor	aRR	Low 95% CI	High 95% CI	P value
BPA <sup>a</sup>	0.95	0.82	1.10	.49
Race/ethnicity	1.06	0.75	1.49	.75
Smoking	0.79	0.51	1.24	.31
Baseline AFC <sup>a,b</sup>	1.77	1.38	2.28	< .0001

Note: aRR = adjusted risk ratio; CI = confidence interval.  
<sup>a</sup> Natural log transformed.  
<sup>b</sup> n = 1 with missing AFC excluded.

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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-à-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 composed 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 composed 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 by 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. did not substantially alter our results (aRR = 0.97, 95% CI = 0.89, 1.05; P=.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<sub>2</sub>, and the number of oocytes retrieved during IVF. In addition, we are unable to consider other clinical factors that 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<sub>2</sub> 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<sub>2</sub>, or number of oocytes retrieved, although higher peak E<sub>2</sub> 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<sub>2</sub> 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 yet unidentified sources of nondietary exposure (41–43). Women were asked to fast before 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<sub>2</sub>, or with the number of oocytes retrieved. Bias associated with fasting variability is therefore likely to be toward the null hypothesis.

Another strength of our study is the use of a Coularray detection method following high-performance liquid chromatography 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). More-

over, the possibility for spontaneous deconjugation of glucuronidated BPA to the unconjugated form is not likely in our study given the storage of specimens at –80°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 data set 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<sub>2</sub> level.

Consistent with the clinical IVF protocol, we captured serum E<sub>2</sub> measures across the cycle and were thereby able to assess peak E<sub>2</sub> 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). A previous report indicated minimal if any cross-reactivity with other estrogenic compounds, and thus we do not anticipate interference with E<sub>2</sub> measures by BPA (48). However, we acknowledge this possibility and suggest that if an interference is present, a bias toward a positive association between serum BPA and peak E<sub>2</sub> would result, opposite to the effect we report herein.

We provide preliminary evidence suggesting that BPA exposure may influence the E<sub>2</sub> 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<sub>2</sub> responses to gonadotropin stimulations seen in a clinic IVF population. Additional studies are needed to confirm the effect of BPA on E<sub>2</sub> 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<sub>2</sub> responses to gonadotropin stimulation.

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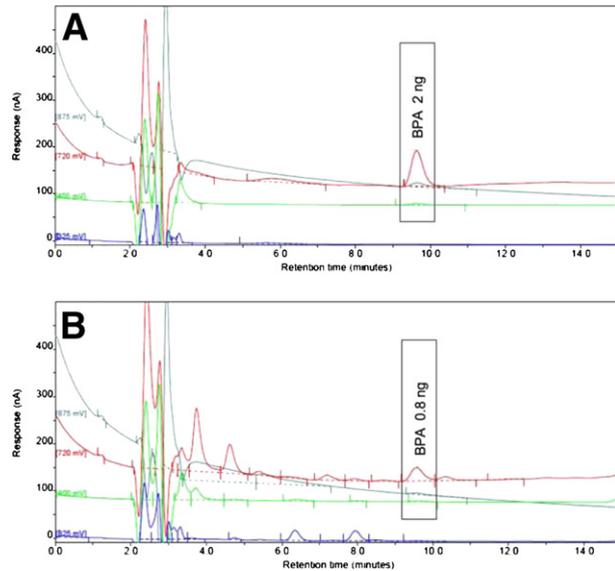
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## SUPPLEMENTAL FIGURE 1

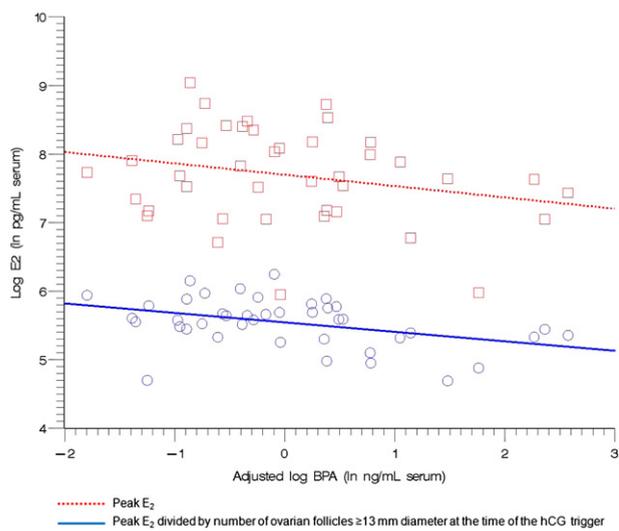
Representative chromatograms for bisphenol A (BPA) analysis in (A) a 2-ng BPA on column standard, and (B) a human serum specimen with 0.8 ng on column using high-performance liquid chromatography with CoulArray detection.



*Bloom. Serum bisphenol A and estradiol response. Fertil Steril 2011.*

## SUPPLEMENTAL FIGURE 2

Association between covariate-adjusted serum unconjugated bisphenol A (BPA) concentrations and peak estradiol ( $E_2$ ) levels measured in 42 women undergoing in vitro fertilization, and participating in the Study of Metals and Assisted Reproductive Technologies (SMART). (Adjusted for antral follicle count (AFC) at baseline, race/ethnicity, and cigarette smoking;  $n = 1$  with missing peak  $E_2$  excluded and  $n = 1$  with missing AFC excluded. hCG = human chorionic gonadotropin.)



Bloom. Serum bisphenol A and estradiol response. *Fertil Steril* 2011.