

Serum unconjugated bisphenol A concentrations in women may adversely influence oocyte quality during in vitro fertilization

Bisphenol A (BPA) is an endocrine disruptor with estrogenic properties that can adversely affect meiotic spindle assemblies. Our data indicate that BPA exposure in female patients may interfere with oocyte quality during IVF, as suggested by the inverse association between serum unconjugated BPA concentration and normal fertilization. (Fertil Steril® 2011;95:1816–9. ©2011 by American Society for Reproductive Medicine.)

Key Words: Bisphenol A (BPA), in vitro fertilization (IVF), oocyte maturation, fertilization, infertility

Bisphenol A (BPA) is gaining global attention as an environmental contaminant of human health relevance owing to its widespread exposure and endocrine-disrupting properties (1, 2). Although knowledge gaps exist regarding the inadvertent BPA exposure of human oocytes, Hunt et al. (3, 4) suggested a relationship between BPA and meiotic disruption of both adult and fetal murine oocytes. BPA has been detected in human follicular fluid, providing evidence that human oocytes are exposed during folliculogenesis (5, 6). Moreover, an inverse association was reported between urinary BPA levels and number of oocytes retrieved and peak estradiol (E₂) levels during in vitro fertilization (IVF), but without comment on oocyte quality (7). To address the dearth of evidence implicating BPA as a disruptor of human oocyte development, we measured serum BPA levels

in women undergoing IVF and correlated these levels with oocyte maturation and fertilization outcomes.

This study cohort has been previously detailed (8) and consists of 58 infertile female patients and 37 male partners undergoing a first IVF cycle at the University of California at San Francisco (UCSF) Center for Reproductive Health between September 1, 2007, and August 31, 2008. Women and men received infertility evaluations, including medical and reproductive histories, and their informed consents were obtained before participation. The study protocol was approved by the UCSF Committee for Human Research.

Female participants underwent gonadotropin-induced ovarian stimulation per clinic protocols. When at least two follicles measured 17 mm in diameter, human chorionic gonadotropin (hCG) (5,000–10,000 IU) was administered subcutaneously and oocytes retrieved 36 hours later. A fasting blood specimen was obtained from women at the time of oocyte retrieval, whereas men, when available, provided a nonfasting blood specimen. Specimens were collected into serum separator Vacutainer tubes (Becton Dickinson and Co., Franklin Lakes, NJ) and allowed to stand for 10 minutes. Serum was aliquoted into 1.8-mL polypropylene cryovials and immediately frozen at –80°C. Oocytes were fertilized by conventional insemination or by intracytoplasmic sperm injection (ICSI) using fresh sperm from male partners or frozen sperm from a male partner or donor. Approximately 16–18 hours after insemination, zygotes were identified by the appearance of two pronuclei. For each ICSI patient, the proportion of mature oocytes collected was defined as the total number of oocytes in metaphase II (MII) arrest divided by the total number of oocytes collected from a patient. The proportion of oocytes fertilized comprised: (1) the total number of zygotes formed divided by the total number of mature oocytes injected from ICSI patients; and (2) the total number of zygotes formed divided by the total number of oocytes with a visible polar body from conventional insemination patients. To maximize statistical power the denominators were collapsed and a single value generated.

Serum was shipped on dry ice to the Endocrine Disruptors Laboratory at the University of Missouri (Columbia, MO) in September 2008. Serum specimens of ≥ 1–2 mL (44 women and

Victor Y. Fujimoto, M.D.^a
Dongsul Kim, B.S.^b
Frederick S. vom Saal, Ph.D.^d
Julie D. Lamb, M.D.^a
Julia A. Taylor, Ph.D.^d
Michael S. Bloom, Ph.D.^{b,c}

^a Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, California

^b Department of Environmental Health Sciences, University at Albany, State University of New York, Rensselaer, New York

^c Department of Epidemiology and Biostatistics, University at Albany, State University of New York, Rensselaer, New York

^d Division of Biological Sciences, University of Missouri, Columbia, Missouri

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Reprint requests: Victor Y. Fujimoto, M.D., Department of Obstetrics, Gynecology, and Reproductive Sciences, 2356 Sutter Street, J707, San Francisco, CA 94115-0916 (E-mail: fujimotov@obgyn.ucsf.edu).

31 men) were assayed for concentrations of unconjugated BPA by high-performance liquid chromatography, using known standards, with an ESA Coullarray 5600 detector (ESA, Chelmsford, MA), as described in detail elsewhere (9). Recoveries averaged 89%, and the limit of detection (LOD) was 0.3 ng/mL in serum. The laboratory was blinded to all clinical and end point data, and machine-read values were reported for samples below the LOD based on extrapolation from the standard curve; these are different from samples for which there was no evidence for the presence of BPA, which were assigned zero values by the machine (10).

Log binomial regression models (11) were used to estimate adjusted associations between log-transformed serum BPA concentrations and oocyte maturity for 31 women undergoing ICSI and fertilization for 26 IVF couples; only participants with complete covariate data were included. Removal of the cumulus mass from retrieved oocytes during ICSI facilitated direct visualization of oocytes before insemination, thereby minimizing misclassification of MII arrest in the analysis of this end point. Age, race/ethnicity, and cigarette smoking were included as covariates contingent on associations with BPA exposure and IVF end points reported in the literature (12, 13). One couple with a missing value for smoking was excluded. The individual oocyte was used as the unit of analysis, and generalized estimating equations were used to accommodate correlations among measures within couples (14). A forward stepwise selection procedure was used to retain statistically significant interactions between serum BPA concentrations and covariates in each model where $P < .05$, augmented by consideration of the quasiliikelihood information criterion. Regression coefficients and 95% confidence intervals (CIs) were exponentiated to generate adjusted relative risks (aRRs) associated with a doubling of BPA concentrations. Statistical analysis was conducted using SAS v.9.2 (SAS Institute, Cary, NC).

The demographics of the study population have been previously described (8). In brief, an average of 13.1 ± 7.8 oocytes were collected during each of 59 initiated study cycles (i.e., only the male

partner participated for one cycle), 0.77 ± 0.22 of which were in MII arrest and 0.64 ± 0.27 of which were fertilized. The median BPA concentrations were 2.53 ng/mL for women (range 0.0–67.4, 86.4% >LOD) and 0.34 ng/mL for men (range 0.0–22.7, 51.6% >LOD), respectively. The results of the multivariable statistical analysis are presented in Table 1. There was no association between BPA and oocyte maturation when all cases were considered (aRR 1.01, 95% CI 0.98–1.05; data not shown). However, in ICSI-only cases, we found a 9% decrease in the probability for a mature oocyte for a doubling of female serum BPA concentration, but only among the nine Asian women (aRR 0.91, 95% CI 0.83–1.00). Moreover, a 55% decrease in the probability for fertilization among the 26 cases of ICSI or conventional insemination was associated with a doubling in female serum BPA concentration (aRR 0.45, 95% CI 0.21–0.66), an effect which was reduced by 2% for each year increase in female age (aRR 1.02, 95% 1.01–1.03) and further reduced by 6% for each doubling of male BPA (aRR 1.06, 95% CI 1.02–1.10). A doubling in male serum BPA concentration was itself associated with a 12% reduction in the probability for fertilization, but only for the five Asian men.

This dataset represents the first evidence in humans that inadvertent BPA exposure may negatively affect oocyte development potential. Although the findings are preliminary, inverse associations detected between BPA in Asian women and oocyte maturity and between BPA in all women and normal fertilization implicate BPA as having a disruptive influence on oocyte developmental competence and oocyte quality.

We can only speculate on the mechanisms responsible for these suggested effects of BPA on human oocytes. It is well established that follicle-stimulating hormone (FSH) interacts synergistically with E_2 to increase luteinizing hormone (LH) receptor and steroidogenic enzyme expression in granulosa cells (15–17). BPA has been demonstrated to bind and activate estrogen receptor (ER) isoforms α and β (18–20). The first indication of an adverse effect of BPA on oocyte development described

TABLE 1

Log-binomial regression models describing the adjusted relative risk (aRR) for a doubling in serum unconjugated bisphenol A (BPA) concentrations on oocyte maturity and fertilization during IVF.

Predictor variable	Model for oocyte maturity ^a				Model for oocyte fertilization ^b			
	aRR	Low 95% CL	High 95% CL	P value	aRR	Low 95% CL	High 95% CL	P value
BPA-female (ng/mL serum)	1.03	0.96	1.10	.428	0.45	0.31	0.66	<.0001
BPA-male (ng/mL serum)	—	—	—	—	0.96	0.88	1.04	.308
Age-female (y)	0.99	0.97	1.01	.242	0.98	0.95	1.02	.261
Age-male (y)	—	—	—	—	0.96	0.94	0.99	.008
Race-female (not Asian/Asian)	1.32	1.04	1.66	.020	1.17	0.70	1.97	.547
Race-male (not Asian/Asian)	—	—	—	—	1.25	0.75	2.08	.386
Smoking-female (never/ever)	1.10	0.92	1.32	.274	1.15	1.03	1.28	.014
Smoking-male (never/ever)	—	—	—	—	0.82	0.69	0.98	.028
BPA-female × race-female	0.91	0.83	1.00	.049	—	—	—	—
BPA-female × age-female	—	—	—	—	1.02	1.01	1.03	<.0001
BPA-female × BPA-male	—	—	—	—	1.06	1.02	1.10	.001
BPA-male × race-male	—	—	—	—	0.88	0.79	0.98	.022

Note: CL = confidence limit; — = not included in the relevant log-binomial regression model.

^a n = 31 women undergoing intracytoplasmic sperm injection (ICSI).

^b n = 26 couples undergoing ICSI or conventional IVF.

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a dramatic increase in meiotic abnormalities as a direct result of coincidental BPA exposure (3). It has been demonstrated that BPA altered murine oocyte development by inducing greater meiotic arrest at either the germinal vesicle (GV) stage or the metaphase I stage, but only at the highest exposure concentration of 30 $\mu\text{mol/L}$ (21). Interestingly, the highest rate of MII abnormalities in that study were found at the lowest BPA dose tested (3 nmol/L), suggesting nonlinear effects (21). Our preliminary dataset focused on human oocyte developmental competence but did not include nondisjunction end points.

Estrogen receptor β is the predominant estrogen receptor found in granulosa cells at all stages of follicular development (22). A functional ER β is necessary for adequate LH-induced cyclic adenosine monophosphate-mediated ovulation occurring via induction of the LH receptor (23–25). That we detected a significant negative association between normal fertilization and female serum BPA concentrations suggests that oocyte quality is impaired to some degree by the presence of BPA (26). However, our limited dataset prevented confirmation of the negative association between normal fertilization and female BPA concentrations owing to statistical instability when only ICSI cases were considered. Although it is not known if meiotic markers are adversely influenced by BPA in humans, our data provide preliminary evidence that cytoplasmic competence and meiotic progression may be affected by BPA. Based on the current literature, it is interesting to speculate that our observations may have occurred due to BPA disruption of ER β -mediated LH receptor induction.

The small number of participants in this study precluded conclusive evaluation of the associations detected between BPA and

oocyte quality, and furthermore, it restricted the number of covariates for which these associations could be adjusted and for which effect modification could be evaluated. Moreover, to maximize our sample size for the fertilization end point, we treated cases of ICSI and conventional insemination as a homogeneous group. This approach may have biased the results if misclassification occurred in the identification of MII arrest in cases of conventional insemination. Another limitation is that BPA was the only environmental exposure considered in the statistical analyses. It is possible that exposures including other phenols may have interactions with BPA effects on oocyte development. We consider one significant advantage of these data to be the measurements of unconjugated serum BPA, rather than total BPA, which includes both conjugated and unconjugated BPA. Thus, we were measuring only the circulating bioactive BPA component. It is not clear if other exposure assessment approaches of BPA would have similar findings, given the variability in BPA metabolism in humans (2).

This preliminary study suggests an overall negative association between BPA exposure at oocyte retrieval and the developmental potential of human oocytes. The complexity of statistical interactions in our regression models suggests that the effects of female BPA are modified by other factors, such as female age, race/ethnicity, and male BPA. Further studies are needed to confirm our findings and elucidate the biologic mechanisms mediating the effects of BPA on human oocyte development during folliculogenesis.

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