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The Association of Bisphenol-A Urinary Concentrations with Antral Follicle Counts and Other Measures of Ovarian Reserve in Women Undergoing Infertility Treatments.☆

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Abstract

In this prospective cohort of women undergoing infertility treatments, we measured specific-gravity adjusted urinary BPA (SG-BPA) concentrations and used regression models to evaluate the association of BPA with antral follicle count (AFC), day-3 serum follicle stimulating hormone levels (FSH), and ovarian volume (OV). BPA, detected in >80% of women, had a geometric mean (±GSD) of 1.6±2.0, 1.7±2.1, and 1.5±1.8μg/L for the women contributing to the AFC (n=154), day-3 FSH (n=120), and OV (n=1 14) analyses, respectively. There was an average decrease in AFC of 12% (95% CI: −23%, −0.6%), 22% (95% CI: −31%, −11%), and 17% (95% CI: −27%, −6%), in the 2nd, 3rd, and 4th SG-BPA quartile compared to the 1st quartile, respectively (*p*-trend:

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

<0.001). No association of SG-BPA with FSH or OV was observed. Among women from an infertility clinic, higher urinary BPA concentrations were associated with lower AFC, raising concern for possible accelerated follicle loss and reproductive aging.

Keywords

Bisphenol-A; Antral Follicle Count; Day-3 Follicle Stimulating Hormone; Ovarian Volume; Ovarian Reserve

1. INTRODUCTION

Endocrine disrupting compounds (EDC) can mimic or antagonize the action of steroid hormones and alter steroid signaling, thus contributing to adverse health outcomes and disease onset. Human and animal data suggest that EDCs might have an adverse impact on reproductive health (1). Indices of human reproduction have been declining over the last several decades, and although delayed childbearing and lifestyle factors have been identified, it is possible that environmental exposure to EDCs may also contribute (1).

Among the EDCs of concern is the estrogenic chemical bisphenol-A (BPA), which has been linked to a broad range of health effects in experimental animal studies (2–6). Exposure to BPA can occur through ingestion, inhalation, and dermal absorption. Bisphenol-A has been extensively used in commerce in the production of polycarbonate plastics, epoxy resins for the interior lacquer coating of food and beverage cans, water pipes, thermal receipt paper, cigarette filters, and some dental products (sealants and composites) (7–8). Repetitive exposure of certain BPA-containing products to light and heat, contact with cleaning agents, and aging of the product may result in increased leaching of BPA into food or beverages (9–11). There is documented widespread human BPA exposure via multiple sources (7, 12–15). BPA has been detected in various biologic fluids including urine, serum, saliva (12, 16), follicular fluid (17), breast milk (18), umbilical cord blood, and amniotic fluid (19–20). Recent evidence suggests that catheter use during delivery may introduce BPA to the mother’s body (21).

Bisphenol-A has structural similarities to the synthetic estrogen diethylstilbestrol and its weak estrogenic properties in vitro are well described (14–15, 22–24). In vivo studies in experimental animals have demonstrated adverse effects of BPA on the onset of puberty, estrus cyclicity, growth and differentiation of the mammary gland and various other reproductive tract targets (25–30). Furthermore, there is evidence that BPA has adverse effects on the maturing oocyte and meiotic cell division machinery (31–33). Consequently, these findings raise concerns that widespread and frequent human exposure to BPA may have an adverse impact not only on the survival of the oocyte but also on the follicular complement, follicle dynamics, ovarian reserve, and fertility in general.

Given that the decline in natural fertility in women is for the most part a silent process, a plethora of tests and biomarkers -to include among others basal follicle stimulating hormone levels (FSH), basal inhibin-B, the ovarian antral follicle count (AFC), and serum levels of antimüllerian hormone (AMH) - became available to assess ovarian function and accurately

identify women with decreased ovarian reserve. Although such tests are frequently labeled as “ovarian reserve tests”, most are better described as “ovarian response tests”, with only AFC and AMH accurately predicting ovarian primordial follicle numbers and appropriately considered “ovarian reserve tests” (34). Furthermore, AFC has been found to be independently associated with natural menopause (35) and appears to be more sensitive than AMH in predicting ovarian primordial follicle numbers (34) and response to medication in IVF cycles (36).

In an effort to better understand the possible effects of BPA exposure on ovarian reserve, we elected to evaluate the association between urinary BPA concentrations and antral follicle counts among women undergoing fertility treatments. Other less sensitive ovarian reserve predictors [such as day-3 FSH and ovarian volume (OV)] were available and thus were included in the analysis.

2. MATERIALS AND METHODS

2.1. Ethical Approval

The study was approved by the Human Studies Institutional Review Boards of Massachusetts General Hospital (MGH), Harvard School of Public Health (HSPH), and the CDC. Participants signed an informed consent prior to study enrollment.

2.2. Study Population

All women enrolled in the current study were recruited from an ongoing study evaluating the relationship between environmental exposures and reproductive health (EARtH study: information can be found at the HSPH website at http://www.hsph.harvard.edu/research/earth/index.html.

Between November 2004 and October 2010, 430 women undergoing infertility treatments at the MGH Fertility Center were recruited in the EARtH study. Of those, only women who had urine samples collected prior to determination of the AFC and the other ovarian reserve parameters were considered for the current analysis (209 unique subjects). Women who had an oophorectomy were excluded from the present analysis due to a possible effect of the surgical intervention on the total AFC.

2.3. Collection of Serum Samples/Ultrasonographic Determinations

All study participants underwent a standard infertility work-up which included measuring serum levels of day-3 FSH and the ultrasonographic determination of the AFC. The transvaginal ultrasounds for the determination of the AFC were performed by one of the MGH fertility physicians. All ultrasounds were performed on the 3rd day of an unstimulated menstrual cycle. No fertility medications were used in the cycle preceding the AFC determination. The OV in mm^3 was calculated using the following formula: *length (mm) x width (mm) x height (mm)* x ($\pi/6$), (37). Day-3 FSH was measured at the MGH Core Laboratory with an automated electrochemiluminescence immunoassay using the Elecsys FSH reagent kit and the Roche Elecsys 1010/2010 immunoassay analyzer (Roche diagnostics, Indianapolis, IN, USA).

2.4. Collection of Urine Samples

A single spot urine sample was collected upon entry into the study and at subsequent treatment cycle visits to the MGH Fertility Center (days 3–9 of the monitoring phase, and at the time of oocyte retrieval or intrauterine insemination). All urine samples included in the current analysis were collected prior to the determination of the AFC and the other ovarian reserve parameters. Urine was collected in a sterile polypropylene cup. Specific gravity (SG) was measured in each sample at room temperature using a handheld refractometer (National Instrument Company Inc, Baltimore, MD, USA) calibrated with deionized water prior to each measurement. Urine SG was measured to allow adjustments for urine dilution. SG was chosen over creatinine concentrations because the latter can be confounded by other parameters such as diet, muscle mass, physical activity, co-existing medical conditions, time of the day, and urine flow (38–39). Subsequently, samples were divided into aliquots and frozen at −80°C. All aliquots were shipped on dry ice to the CDC, where they remained frozen at or below −40°C until final analysis. Urinary BPA concentration was measured using online solid phase extraction coupled to HPLC-isotope dilution tandem mass spectrometry, as previously described (40). The limit of detection (LOD) was 0.4μg/L.

2.5. Statistical Analysis

The urinary BPA concentrations were log-normally distributed. Therefore, for each woman the geometric mean of the BPA concentrations from all available urine samples collected prior to the date of the ovarian reserve measure was calculated and used as the woman's summary BPA concentration. BPA concentrations <LOD were assigned a value equal to LOD divided by the square root of two (41). Urinary BPA concentrations (μg/L) were adjusted for SG in all analyses using a modification of a previously described formula (42): BPAc = BPAm [(1.016 – 1)/SG - 1], where BPAc is the SG-corrected BPA concentration (μg/L), BPAm is the measured BPA concentration, and 1.016 is the median SG level in the study population. Because not all outcome measures were available for all participants, three separate datasets were created (AFC, day-3 FSH, and OV datasets). In 44 women (20%), all 3 outcomes were available for analysis. These women were included in all three datasets. In 91 women two outcomes were available, and the rest of the women had only one ovarian reserve parameter available for evaluation. Poisson regression models were used to analyze the association between urinary BPA concentrations (divided into quartiles) with AFC. Multivariable linear regression was used to evaluate the association between urinary BPA concentrations (divided into quartiles) with day-3 FSH and with OV (log transformed to reduce skewness). Covariates considered for inclusion in the regression models included age and body mass index (BMI). In order to further evaluate the association between urinary BPA quartiles and AFC, we used a cut-off level of ≥15 antral follicles (to distinguish between high- and normal/low-responders), and calculated odds ratios using logistic regression, adjusted for age. The rationale for choosing this cut-off is based on the fact that the median oocyte yield in our IVF practice is 10.0 (25^{th}, 75^{th} percentile: 7.0 and 14.0 oocytes, respectively) and less than ¼ of the women in our IVF practice have more than 15 oocytes retrieved. Furthermore, in sensitivity analyses, regression models for AFC and OV were rerun excluding patients diagnosed with polycystic ovarian syndrome (PCOS), since these patients often have very high AFC (43–44). All statistical analyses were conducted

using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Two-sided significance levels <0.05 were considered statistically significant.

3. RESULTS

Urinary BPA concentrations were measured in 209 women from the infertility clinic. AFC, day-3 FSH serum levels, and OV were available for 154, 120, and 114 women, respectively. The demographic characteristics of the study population were summarized in Table 1. The population was predominantly Caucasian with a mean age of 36.0 years (range: 21.6 – 46.7), and a mean BMI of 25.3 kg/m^2 (range: 16.5 – 42.4). Most patients were never smokers and the most common infertility diagnosis was male factor followed by idiopathic infertility.

Table 2 summarizes the distribution of specific gravity (SG)-adjusted urinary BPA concentrations using subject-specific summary exposure measures. Unadjusted urinary BPA concentrations were also included in the same table to allow comparisons with other studies. Urinary BPA concentrations were measured in 360, 330, and 267 urine samples among women included in the AFC, day-3 FSH, and OV datasets, with geometric means ranging from 1.5 to 1.7 μg/L (Table 2). The unadjusted concentrations were comparable to those previously reported in the general US population [2.6 μg/L, (45)]. 44% or more of study participants contributed at least two urine samples (46%, 44%, and 45% for the AFC, day-3 FSH, and OV datasets respectively), and 14% or more contributed at least five urine samples (20%, 14%, 15% for the day-3 FSH, OV, and AFC datasets respectively). Among all urine samples, 15–18% had BPA concentrations below the LOD (Table 2).

Age was significantly related to all of the outcome measures (p <0.05) and was included as a covariate in all regression models as a continuous measure, whereas BMI was not associated and thus was not included (univariate analysis p-values for the association of BMI with AFC, FSH, OV were: 0.43, 0.43, and 0.51 respectively).

3.1. BPA and Antral Follicle Count

Controlling for age, there was a significant trend towards lower AFC with higher urinary BPA quartiles (p-trend: < 0.001). In the 2nd, 3rd, and 4thquartile as compared to the 1stquartile, there was an average decrease of 12% (95% CI: −23%, −0.6%, p: 0.04), 22% (95% CI: −31%, −11%, p: 0.0002), and 17% (95% CI: −27%, −6%, p=0.0035), in the total AFC, respectively (Table 3), (supplemental Figure 1). The results were similar when patients with PCOS were excluded from the analysis; after controlling for age, there was a significant trend towards lower AFC with higher urinary BPA quartiles (p-trend: 0.0029). In the 3rd and 4th quartile as compared to the 1st quartile, there was an average decrease of 16% (95% CI: −27.0%, −3.5%, p=0.014), and 17% (95% CI: −28.0%, −4.7%, p=0.0089) respectively, in the AFC. While AFC for the 3rd and 4th quartiles were significantly lower than the 1st quartile (p < 0.01 for each comparison), there was a non-significant 6.0% (p=0.40) decrease in the 2nd compared to the 1st quartile (Table 3).

In logistic regression models comparing high versus normal/low responders, women with SG-BPA urinary concentrations in the 3rd quartile had increased odds of having an AFC<15 [3rd quartile: OR=3.66 (95% CI: 1.13, 11.87), 4th quartile: OR=2.23 (95% CI: 0.76, 6.57),

see Table 4, supplemental Figure 2]. Overall, a suggestive trend for increased odds of having an AFC<15 was noted with increasing BPA urinary concentration quartiles (*p*-trend: 0.077). The trend reached significance when excluding patients with PCOS (*p*-trend: 0.029).

3.2. BPA, Day-3 FSH, and Ovarian Volume

There were no significant associations between quartiles of BPA urinary concentrations and either day-3 FSH serum levels (*p*-trend: 0.64) or OV (*p*-trend: 0.8) based on age-adjusted linear regression models (supplemental Tables 5 and supplemental Tables 6). Controlling for age there was an average decrease of 0.66 (*p*: 0.41), 0.07 (*p*: 0.93), and 0.85 IU/L (*p*: 0.29) in day-3 FSH in the 4^{th}, 3^{rd}, and 2^{nd} quartile of SG adjusted urinary BPA concentrations compared to the 1^{st} quartile, respectively. For ovarian volume, after controlling for age there was a 0.39% (*p*: 0.98), 7.81% (*p*: 0.59), and 21.1% (*p*: 0.12) decrease in average ovarian volume in the 4^{th}, 3^{rd}, and 2^{nd} quartile of SG-adjusted urinary BPA quartiles compared to the 1^{st}quartile, respectively.

4. DISCUSSION

The BPA urinary concentrations among women in our study were comparable to those previously reported for the US general population (45). In the present study, there was a significant trend towards lower AFC with higher urinary concentration of BPA, suggesting that exposure to environmental levels of BPA might have an adverse impact on ovarian function in this group of women. The hypothesis that common and relevant environmental exposures affect ovarian age is not new. Smoking has been linked to several indicators of ovarian age (follicle number, FSH, age at menopause) and adverse reproductive outcomes (delayed conception and increased spontaneous abortion rates), while chronic exposure to dioxins hastens reproductive aging by disrupting the local ovarian endocrine environment (46–50). Our study, the first we are aware of examining the effects of a ubiquitous environmental chemical on ovarian age indicators, suggests that exposure to BPA might have adverse effects on ovarian age as well.

Although it would be of importance to identify the mechanisms through which BPA may adversely impact oocyte viability, the current study was not designed to determine this. However, other published studies with results relevant to ours have identified possible reproductive targets of BPA and will therefore be discussed in an attempt to explain our findings and elucidate the potential mechanisms underlying BPA's actions. In doing so, we recognize that the task of identifying potentially toxic exposures and quantifying their cumulative effect on reproductive targets can be extremely difficult, because the oocyte pool consists of a non-regenerating population of cells, one of the longest-lived in the human body, whose formation starts prior to birth and depends on both genetic factors and a fine balance between estradiol and local paracrine factors (1; 51–52). The fate of the oocyte pool in adult life alike depends on multiple factors (including environmental chemicals) that can accelerate the genetically predetermined rate of oocyte loss thus depleting the follicular pool and adversely impacting the female's fertile life span.

Theoretically, BPA could act by targeting the oocyte or its supporting ovarian environment either in fetal or in adult life. In that regard, there is evidence in laboratory animals

suggesting that BPA can do both. Fetal exposure to BPA and EDCs can modify the starting oocyte pool through mechanisms involving altered gene expression, modified neuroendocrine signaling, and epigenetics (1), and thus potentially disrupts early follicular development, and produces quantitative and qualitative oocyte changes that might not become detectable until adulthood (1, 53). In one study, the BPA-induced disruption of oocyte development in fetal life had multigenerational consequences with female mice producing an increased number of chromosomally abnormal eggs and embryos in adult life (54). In another, prenatal exposure to BPA induced postnatal changes consistent with reproductive aging (formation of blood-filled ovarian bursae in 6-month-old CD-1 mice) (55). The above studies, although evaluating exposures at a time period different than during adult life as in the present study, provide evidence that exposure to EDCs that can mimic or antagonize the effects of estrogen may have an adverse and direct impact on oocyte development.

The follicular pool decrease noted in our study could have resulted from similar mechanisms either directly affecting the oocyte or indirectly affecting the local, intra-ovarian and intra-follicular environment (i.e., by affecting the granulosa cell lineage that nourishes and matures the oocyte throughout life). It is known that various other factors (including environmental toxicants, chemotherapeutics, and radiation) can accelerate the rate of follicular depletion through atresia, resulting in wastage of oocytes, thus shortening the time to reproductive aging and menopause.

In regards to the possibility of an adverse effect of BPA on the supporting granulosa cell line, there is *in vitro* evidence supporting this. Granulosa cells are found in large numbers in the ovary, constitute an indispensable component of ovarian folliculogenesis and steroidogenesis and play a pivotal role in the survival of the oocyte pool. Granulosa cell exposure to BPA decreased murine granulosa cell viability, increased G2-to-M arrest in a dose- and time-dependent manner and altered the balance of anti-apoptotic and pro-apoptotic proteins, thus favoring apoptosis (56). BPA can further affect oocyte viability by antagonizing the anti-apoptotic effects of certain hormones (i.e., intrinsic estradiol), effects critical for the survival of the granulosa cell line (57). Biochemical assays have determined that BPA can bind to both estrogen receptors alpha and beta, and thus can exert an estrogenic action (58–59). Therefore, BPA, through binding to the ERα, might antagonize the anti-apoptotic effects of estrogens (22, 56). The BPA medicated disruption of key ovarian steroidogenic enzymes can further decrease FSH-induced estradiol production by the granulosa cells, thus increasing apoptosis (22, 60). Treatment of FSH-stimulated porcine granulosa cells with BPA resulted in a significant inhibition of estradiol production (60), while a significant concentration-dependent inhibitory effect of BPA on estradiol levels and the expression of P450arom mRNA has been observed in rat ovarian granulosa cells (61). Similarly, mouse oocytes exposed in vitro to BPA during follicular development showed reduced granulosa cell proliferation and a lower estrogen production (17), while the granulosa cells exposed to BPA were darker and rounded up, both signs of reduced health. Similarly, Peretz et al provided further evidence suggesting that postnatal exposure to BPA targets the estradiol biosynthesis pathway in the ovary and inhibits antral follicle growth (62). All of the above findings support the biological plausibility of the results of the current

study demonstrating lower AFC counts with increased urinary concentrations of BPA. Furthermore, our results are consistent with previously published results from women undergoing IVF demonstrating a negative association of urinary BPA concentrations with both the number of oocytes retrieved per cycle and the peak serum estradiol levels (63) and a positive linear dose-response association between BPA concentrations and implantation failure (64–65).

Finally, there is evidence suggesting a direct, adverse effect of BPA on the maturing oocyte. Hunt et al, reported failure of chromosomes to properly align at the spindle equator in metaphase II mouse oocytes accidentally exposed to BPA and a significant dose-related increase in the percent of oocytes with congression failure was noted with increasingly higher oral doses of BPA (31). Further evidence of the adverse effects of BPA on the maturing oocyte and the meiotic cell division machinery was provided by Can et al, who noted a delay in the meiotic cell cycle and abnormalities in the chromosome alignment on the meiotic spindle, likely resulting from degradation of centrosomal proteins and alterations in the structural integrity of the meiotic spindle microtubules (32). They postulated that BPA is more potent in gametes than in somatic cells and may be considered a reproductive toxicant. Results from other more recent experiments on mouse oocytes suggest that the disrupting effects of BPA on the meiotic spindle formation might lead to the death of the oocyte rather than aneuploidy (33). Finally, a similar detrimental effect of BPA on the survival of the human oocyte was provided by Brieno-Enriquez et al who noted that addition of BPA to the culture medium decreased the survival of human fetal oocytes that degenerated at rates five times higher than without BPA, an effect possibly mediated via a tissue-specific, estrogenic receptor-related function (66).

Furthermore, BPA increased the percentage of oocytes at leptonema and reduced the percentage of oocytes that reached pachynema in vitro, independently of the concentration used thus affecting meiotic progression of exposed oocytes.

In conclusion, our finding of lower AFC with higher urinary concentrations of BPA is in agreement with the toxicological literature evaluating possible adverse effects of BPA on the oocyte and its supporting environment in fetal and adult life alike. Although the design of our study does not allow us to investigate the possible mechanisms mediating this effect, our study provides evidence for a possible adverse effect of BPA on the human oocyte and the supporting follicular environment.

In regards to a possible association of BPA with either day-3 FSH or OV, we found no evidence to support this. The three ovarian reserve indicators evaluated in this study were each associated, in the expected direction, with each other and with the woman’s age. Because the day-3 FSH and OV datasets contained fewer patients than the AFC dataset it is possible that the smaller number did not provide adequate power for meaningful statistical conclusions. AFC is not as sensitive to monthly fluctuations as the FSH is and might therefore be a more sensitive indicator of the ovarian reserve. Studies suggest that the marker best reflecting the “true” ovarian reserve (histologically confirmed ovarian primordial follicle number) is the ultrasonographically determined antral follicle count (34), with FSH either showing a weaker correlation or none at all (after adjusting for age,

significant correlations were identified between the ovarian primordial follicle count and AFC and AMH only). In regards to “operator” related differences, the ultrasonographic determination of the AFC is more “standardized” in our center than that of the ovarian volume and therefore most probably had less inter-operator variability.

To the best of our knowledge, our study is the first to explore the possible association of BPA with ovarian reserve in the human. The study has many strengths. It was performed in one center with a diverse patient population and all urine samples were collected and processed under one protocol prior to the determination of the ovarian reserve parameters. All BPA determinations and the vast majority of the FSH measurements were performed each by the same laboratory using the same assay. The ultrasonographic determination of the AFC was performed by infertility specialists only, all working at the same center, and following the same guidelines to minimize “between operators” variability.

The study also has certain limitations, though. First, exposures to many environmental chemicals are episodic, yet chronic, and for short-lived environmental chemicals such as BPA assessing long-term exposure is difficult. We attempted to partially account for this by collecting multiple urine samples that preceded by a few weeks to a few months the ovarian reserve evaluation. More than half of the subjects had 2 or more samples, and up to a fifth had more than 5 urine samples prior to the determination of the ovarian reserve parameters. Also, although we quantified BPA with a multi-analyte method successfully used for the analyses of tens of thousands of samples including those collected as part of the US National Health and Nutrition Examination Survey and considered the gold standard for measuring BPA in people (45), its sensitivity is lower than for other methods described in the literature, thus impacting the detection frequency of BPA in our cohort. Nonetheless, we detected BPA in 82%–85% of the samples, depending on the outcome evaluated, a detection frequency well above the minimum 50% considered necessary for the imputation method (41) we used in our statistical analyses. Another limitation arises from our lack of data from the prenatal period (i.e., exposures, other adverse factors) and the fact that oogenesis is a complex process the origins of which might date back to fetal life. Also, the sites targeted by BPA and critical time periods can be multiple, thus making the assessment of changes in oocyte quantity and quality resulting from such exposures a considerable epidemiologic challenge. In order to create an endocrine environment fostering the survival and growth of the oocyte, complex bi-directional signaling between various strategic sites (to include the ovary, the pituitary, and the hypothalamus) and between the oocyte and its supporting granulosa cell lineage is required. Chronic low or high dose exposure to BPA might interfere with any of these vital interactions and might disrupt the oocyte-granulosa cell crosstalk, eventually pushing the oocyte into the cell death pathway. Another limitation arises from the fact that AMH was not measured during the study period (the AMH assay was not commercially available at our center at the time and the test was neither required nor approved by the insurance). However, AFC has been found to be slightly more sensitive than AMH in predicting ovarian primordial follicle (34) and response to medication in IVF cycles (36). Finally, the results should be interpreted with caution because the findings may not be generalizable to women from the general population (i.e., conceiving spontaneously), coexposures to other select chemicals were not accounted for, and exposure to BPA may be reflective of other unknown lifestyle factors that might be affecting ovarian reserve.

4.1. Conclusions

In conclusion, BPA was detected in the majority of the urine samples collected from women seeking infertility treatments. Furthermore, there was a trend towards lower AFC with higher urinary BPA concentrations. These data support results from in vitro and animal studies and emphasize a possible risk of BPA exposure to human reproductive health, but little information is available regarding the effects of adult BPA exposure on ovarian reserve. Our study provided evidence for a potential association between BPA and reproductive senescence in humans. Additional studies are needed to further elucidate the diverse and complex mechanisms through which BPA may target key reproductive functions thus affecting the reproductive health of women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AFC	Antral Follicle Count
AMH	Anti-Mullerian Hormone
BPA	Bisphenol-A
EDC	Endocrine Disrupting Compounds
FSH	Follicle Stimulating Hormone
HSPH	Harvard School of Public Health
LOD	Limit Of Detection
MGH	Massachusetts General Hospital
OV	Ovarian Volume
SG	specific gravity

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Research Highlights

- Association of urinary BPA concentrations with AFC & other ovarian reserve parameters.
- BPA was detected in over 80% women.
- Higher BPA urinary concentrations were associated with lower antral follicle counts.
- No association of BPA with FSH and OV was observed.

Table 1

Demographic characteristics, SART fertility diagnosis and measures of ovarian reserve, within subgroups defined by available outcomes measures

	Antral Follicle Count N=154	Day 3 FSH N=120	Ovarian Volume N=114
Age (years)			
Mean ± SD	36.1 ± 4.2	36.0 ± 4.6	35.6 ± 4.6
Range	21.0–44.8	22.0–45.3	21.7– 46.7
BMI (kg/m^2)			
Mean ± SD	25.2 ± 5.2	25.4 ± 5.5	24.9 ± 4.7
Range	16.5–40.5	17.3–42.4	17.5–40.5
Race [n(%)][a]			
Caucasian	126(82)	94(78)	96(84)
African-American	6(4)	8(7)	5 (4)
Asian	9(6)	5(4)	8(7)
Native American/Alaska Native	1(1)	1(1)	0
Other	12(8)	12(10)	5(4)
Smoking History [n(%)][a]			
Never	115(75)	94(78)	79(69)
Former	32(21)	22(18)	29(25)
Current	7(5)	4(3)	6(5)
SART Diagnosis [n(%)][a]			
Female Factor	62(41)	50(42)	42(37)
Diminished ovarian reserve	18(12)	15(13)	10(9)
Ovulation Disorders	23(15)	17(14)	18(16)
Tubal Factor	11(7)	9(8)	6(5)
Uterine Factor	1(1)	1(1)	0
Endometriosis	9(6)	8(7)	8(7)
Male Factor	38(25)	33(28)	32(28)
Unexplained	44(29)	29(24)	32(28)
Other	9(6)	7(6)	7(6)
Antral Follicle Count (AFC)[b]			
Mean ± SD	12.3±7.0		
Range	2.0–40.0		
Day 3 FSH (IU/L)			
Mean ± SD		7.4 ±3.2	
Range		0.1–26.0	
Ovarian Volume (mm^3) (OV)			
Mean ± SD			5945.1± 3727
Range			1,276– 27,834

[a] Due to rounding, percents may not equal 100%,

[b] Antral follicle count summation of left and right ovary counts.

Table 2

Distribution of urinary BPA (μg/L) among 209 women, within subsets defined by available outcomes measures

	N[a]	% < LOD[b]	GM (GSD)[f]	Median (25th, 75th percentile)	Maximum
Antral Follicle Count					
BPA	154	17[c]	1.3 (2.5)	1.2 (0.7, 2.3)	30.8
SG-adjusted BPA	154		1.6 (2.0)	1.6 (0.9, 2.3)	20.5
Day 3 FSH					
BPA	120	15[d]	1.5 (2.5)	1.6 (0.8, 2.4)	16.8
SG-adjusted BPA	120		1.7 (2.1)	1.7 (1.0, 2.6)	13.3
Ovarian Volume					
BPA	114	18[e]	1.2 (2.4)	1.3 (0.6, 2.3)	11.3
SG-adjusted BPA	114		1.5 (1.8)	1.6 (1.1, 2.4)	6.8

[a] N: Number of women,

[b] Percent of samples below LOD: limit of detection (LOD = 0.4 μg/L),

[c] 61 samples out of 360,

[d] 49 samples out of 330,

[e] 48 samples out of 267,

[f] GM: geometric mean (mean of logged concentrations), GSD: geometric standard deviation.

Table 3

Poisson regression model for the association of urinary BPA quartiles with Antral Follicle Count (sum), controlling for age.

	PCOS patients included				PCOS patients excluded			
Quartile	**N**	**Adjusted Mean % Change in AFC**	**95% CI (%)**	***p*-value**	**N**	**Adjusted Mean % Change in AFC**	**95% CI (%)**	***p*-value**
Q4(2.4–20.5 μg/L)	38	−17	−27, −6.0	0.0035	30	−17	−28,−4.7	0.0089
Q3(1.6–2.3 μg/L)	39	−22	−31, −11	0.0002	33	−16	−27, −3.5	0.014
Q2(0.9–1.6 μg/L)	39	−12	−23, −0.6	0.040	30	−6.0	−18, 8.2	0.39
Q1(<0.4–0.9 μg/L)	38	1 (Ref)			34	1 (Ref)		
p-value for trend		0.0008				0.0029		

Table 4

Odds Ratios (OR) for urinary BPA quartiles among women with sum AFC ≥15 compared to women with sum AFC<15, controlling for age.

	PCOS patients included				PCOS patients excluded			
Quartile	**N(Total) AFC<15 AFC≥15**	**OR**	**95% CI**	***p*-value**	**N(Total) AFC<15 AFC≥15**	**OR**	**95% CI**	***p*-value**
Q4(2.4–20.5 μg/L)	38	2.23	0.76, 6.57	0.15	30	3.05	0.85, 10.94	0.088
	28				24			
	10				6			
Q3(1.6–2.3 μg/L)	39	3.66	1.13, 11.87	0.031	33	4.71	1.192, 18.67	0.027
	33				29			
	6				4			
Q2(0.9–1.6 μg/L)	39	1.66	0.59, 4.70	0.34	30	1.25	0.39, 3.97	0.71
	29				22			
	10				8			
Q1(<0.4–0.9 μg/L)	38	1 (Ref)			34	1 (Ref)		
	25				23			
	13				11			
p-value for trend		0.077				0.029		