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# Urinary bisphenol A concentrations and ovarian response among women undergoing IVF

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## Summary

Bisphenol A (BPA) is a synthetic chemical used in the manufacture of materials present in many common consumer products. In experimental animals, BPA caused oocyte aneuploidy and reduced production of oestradiol. In a prospective cohort study, we investigated the association between urinary BPA concentrations and ovarian response among women undergoing in vitro fertilization (IVF) at the Massachusetts General Hospital (MGH) Fertility Center. The geometric mean of two specific-gravity (SG) adjusted urinary BPA concentrations collected during each IVF cycle was used as the cycle-specific BPA exposure level. BPA concentrations were measured using online solid phase extraction coupled to isotope dilution-high-performance liquid chromatography-tandem mass spectrometry. Peak serum oestradiol was measured using the Elecsys Estradiol II immunoassay kit. Multivariable mixed effect models and Poisson regression models adjusting for correlation between multiple IVF cycles in the same woman were used to evaluate the association between urinary BPA concentrations and ovarian response, adjusting for age, BMI and day 3 follicle stimulating hormone (FSH) levels, a clinical measure of ovarian reserve. Urinary BPA concentrations were measured in 84 women (mean age 35.6 years) undergoing 112 IVF cycles; 23 women (27%) contributed more than one IVF cycle. BPA concentrations ranged from <0.4 to 25.5 $\mu$g/L (geometric mean 2.52 ± SD 3.2); 15% of urine samples had concentrations <0.4 $\mu$g/L. Peak serum oestradiol levels correlated with the total number of oocytes retrieved per cycle ($r = 0.65$, $p < 0.001$). For each log unit increase in SG-BPA, there was an average decrease of 12% (95% CI: 4, 23%; $p = 0.007$) in the number of oocytes retrieved and an average decrease of 213 pg/ml (95% CI: −407, −20; $p = 0.03$) in peak oestradiol. BPA was detected in the urine of the majority of women undergoing IVF, and was inversely associated with number of oocytes retrieved and peak oestradiol levels.



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**Disclaimer**

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

**Keywords**

bisphenol A; in vitro fertilization; human oestradiol; oocyte

## Introduction

The potential risk from exposure to environmental toxicants is a current concern amongst the scientific community, policymakers and the general public. Bisphenol A [BPA, 2,2-bis-(4-hydroxyphenyl)-propane] is a synthetic oestrogen that is widely used in the production of polycarbonate plastics for baby and water bottles (Brede *et al.*, 2003), epoxy resins for the lacquer lining of food and beverage cans and water pipes (Bae *et al.*, 2002), and some dental sealants and composites (Sasaki *et al.*, 2005; Joskow *et al.*, 2006). Over time and with repeated exposure to heat (Brede *et al.*, 2003), polycarbonate plastics and epoxy resins can leach BPA into food and liquids.

Over 6 billion pounds of BPA are produced worldwide each year, and the increased use of consumer products based on polycarbonate plastics and epoxy resins (Kang *et al.*, 2006) has led to widespread exposure of the general population to BPA. In the 2003–2004 National Health and Nutrition Examination Survey (NHANES), BPA was detected in 93% of urine samples obtained from 2517 US residents (Calafat *et al.*, 2008). Human studies have measured detectable concentrations of BPA in follicular and amniotic fluid (Ikezuki *et al.*, 2002) and umbilical cord blood (Schonfelder *et al.*, 2002), suggesting that exposure may begin as early as the periconception and prenatal period.

In adult humans, BPA is rapidly metabolized via hepatic glucuronidation and entirely excreted in urine. The biological half-life of BPA following oral ingestion is <6 h, with nearly complete urinary excretion in 24 h (Volkel *et al.*, 2002). Urinary concentrations are therefore reflective of recent BPA exposure. Despite the short half-life, Mahalingaiah *et al.* (2008) found that a single urine sample was moderately predictive of long-term (over weeks or months) urinary BPA concentrations.

Bisphenol A has been known to have oestrogenic activity since 1936 (Dodds & Lawson, 1936), around the time when diethylstilbestrol (DES), another manufactured oestrogen with a similar molecular structure to BPA, was synthesized. DES was widely prescribed until evidence linked antenatal exposure to reproductive tract abnormalities (Herbst & Scully, 1970; Kaufman *et al.*, 1977). Although BPA was never used as a pharmaceutical, chronic low-level exposure to this synthetic oestrogen has raised serious concerns about effects on human development and reproductive health (Vom Saal *et al.*, 2007).

Some of the earliest and most influential work on BPA exposure and female fertility was performed by Hunt *et al.*, who found that in vivo exposure of mice to low doses of BPA resulted in a significant dose-related increase in oocyte aneuploidy (Hunt *et al.*, 2003). In vitro studies have confirmed the ability of BPA to disrupt meiotic spindle formation, centrosome dynamics and chromosome alignment and segregation (Can *et al.*, 2005; Lenie *et al.*, 2008). In addition to oocyte aneuploidy, in vitro studies using porcine and rat ovarian cells showed that exposure to low doses of BPA resulted in significant concentration-dependent inhibition of oestradiol production (Mlynarcikova *et al.*, 2005; Zhou *et al.*, 2008).

The potential clinical implications of BPA-induced oocyte aneuploidy and oestradiol suppression include decreased fertility as a result of poor oocyte maturation, as well as early pregnancy loss from chromosomal abnormalities in the embryo and foetus. Despite the numerous animal studies, there are few studies on BPA exposure and human reproduction.

Given the effects of BPA on aneuploidy and oestrogen production in experimental animals, we performed a prospective cohort study to determine the effect of BPA on human reproduction. Specifically, we designed this study to investigate the association of pre- and peri-conception urinary BPA concentrations with oocyte and oestradiol production among women undergoing in vitro fertilization (IVF).

## Methods

### Subjects

Study subjects were female partners of couples seeking infertility evaluation and treatment at the Massachusetts General Hospital (MGH) Fertility Center. They were recruited between November 2004 and August 2008. Women between ages 18 and 45 years and who used their own oocytes for IVF were eligible; those who used donor oocytes and donor embryos were ineligible. Women who underwent cryo-thaw cycles were also ineligible. Women were followed from study entry throughout each of their IVF treatment cycles until either a live birth was achieved or the discontinuation of treatment at the MGH Fertility Center.

The study was approved by the Human Studies Institutional Review Boards of the Massachusetts General Hospital, Harvard School of Public Health, and the Centers for Disease Control and Prevention (CDC). Subjects signed an informed consent after the study procedures were explained by a research nurse and all questions were answered.

### Questionnaires and examination

A brief questionnaire was administered by research nurses to collect data on demographics, reproductive medical history, and lifestyle factors such as smoking. Subjects were also asked to complete a detailed take-home questionnaire with additional questions on lifestyle factors, diet, and medical history. Height and weight were measured on site by the research nurse.

### Clinical data

Clinical information from the subject's electronic medical record was abstracted by the research nurses. All subjects underwent an evaluation for infertility which included a follicle-stimulating hormone (FSH) level drawn on the third day of the menstrual cycle to assess ovarian reserve. After completion of the standard infertility work-up, each subject was given an infertility diagnosis by their reproductive endocrinologist according to the Society for Assisted Reproductive Technology (SART) definitions. SART diagnoses consisted of male factor infertility which included poor semen quantity/quality; female factor infertility which included endometriosis, diminished ovarian reserve, tubal and uterine disorders; other causes and unexplained infertility.

Upon completion of the infertility evaluation, subjects underwent one of three IVF treatment protocols used at the MGH Fertility Center. The three IVF treatment protocols were: (1) Luteal phase GnRH-agonist protocol using low, regular and high-dose leuprolide (Lupron), in which pituitary desensitization was begun in the luteal phase; (2) Follicular phase GnRH-agonist/Flare protocol, in which Lupron was begun in the follicular phase on day 2 of menses at 20 units and decreased to the standard dose of five units on day 5; and (3) GnRH-antagonist protocol, in which GnRH-antagonist was begun when the lead follicle reached 14 mm in size. All cycles were preceded by a cycle of oral contraceptive pills unless contraindicated. On day 3 of induced menses, exogenous gonadotropins [FSH (Gonal-F, Follistim, Bravelle)] and/or Human Menopausal Gonadotropin [hMG (Repronex, Menopur)] were initiated. In the luteal phase GnRH-agonist protocol, Lupron dose was reduced at, or shortly after, the start of ovarian stimulation with FSH/hMG. FSH/hMG and GnRH-agonist

or GnRH-antagonist was continued to the day of trigger with Human Chorionic Gonadotropin (hCG), 36 h before oocyte retrieval.

### Serum FSH and oestradiol measurements and oocyte retrieval

The day 3 FSH concentration was measured with an automated electrochemiluminescence immunoassay using the Elecsys FSH reagent kit and the Roche Elecys 1010/2010 immunoassay analyser (Roche Diagnostics, Indianapolis, IN, USA) at the MGH Core Laboratory. Serum samples to measure oestradiol were collected throughout the monitoring phase of the subject's IVF treatment cycle. Serum oestradiol was used as a marker of ovarian stimulation and follicular development. The concentration of oestradiol was measured with an automated electrochemiluminescence immunoassay using the Elecsys Estradiol II reagent kit and the Roche Elecys 1010/2010 immunoassay analyser (Roche Diagnostics) at the MGH Core Laboratory. The peak oestradiol concentration was defined as the highest level of oestradiol prior to oocyte retrieval, which was obtained on the day of trigger with hCG.

Oocyte retrieval was performed when follicle sizes on transvaginal ultrasound reached 16–18 mm and the peak oestradiol level reached at least 500 pg/mL. Information on the physician who performed the oocyte retrieval and any complications of the procedure were collected. Retrieved oocytes were cultured in one of two medias: (1) Quinn's Advantage Fertilization Medium (CooperSurgical Inc., Trumbull, CT, USA) or (2) IVC-TWO (InVitroCare Inc., Frederick, MD, USA). Trained embryologists at the MGH Fertility Center identified the total number of oocytes retrieved per cycle.

### Urine sample collection and urinary BPA measurements

Women provided two urine samples per cycle. One sample was collected at the beginning of the cycle on day 3 or day 4 of the gonadotropin phase, and the second sample was collected on the day of oocyte retrieval. Urine was collected in a clean polypropylene container. After measuring specific gravity (SG), the urine was divided into aliquots and frozen at −80 °C. Samples were shipped on dry ice overnight to the CDC where they were stored at or below −40 °C until analysis.

The total urinary concentration of BPA was measured using online solid phase extraction (SPE) coupled to isotope dilution-high-performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS) on a system constructed from several HPLC Agilent 1100 modules (Agilent Technologies, Wilmington, DE, USA) coupled to a triple quadropole API 4000 mass spectrometer (Applied Biosystems, Foster City, CA, USA) (Ye *et al.*, 2005). First, 100 $\mu$L of urine was treated with $\beta$-glucuronidase/sulphatase (*Helix pomatia*, H1; Sigma Chemical Co, St. Louis, MO, USA) to hydrolyse the BPA-conjugated species. BPA was then retained and concentrated on a C18 reversed-phase size-exclusion SPE column (Merck KGaA, Darmstadt, Germany), separated from other urine matrix components using a pair of monolithic HPLC columns (Merck KGaA), and detected by negative ion-atmospheric pressure chemical ionization-MS/MS. The limit of detection (LOD) for BPA was 0.4 $\mu$g/L. In addition to unknown samples, each analytical run included low-concentration and high-concentration quality control materials, prepared with pooled human urine, and reagent blanks to assure the accuracy and reliability of the data (Ye *et al.*, 2005). BPA concentrations <LOD were assigned a value equal to one-half the LOD (Hornung & Reed, 1990) prior to adjustment by SG.

We used SG instead of creatinine to adjust for urine volume as creatinine concentrations may be confounded by muscle mass, physical activity, urine flow, time of day, diet and disease states (Boeniger *et al.*, 1993; Teass *et al.*, 1993). In addition, organic compounds

such as phenols are glucuronidated in the liver and are eliminated by active tubular secretion, so that creatinine adjustment may not be appropriate (Teass *et al.*, 1993). Urinary BPA concentrations were normalized for dilution using the formula Pc = P × [(1.024–1)/(SG–1)], where Pc is the SG-corrected BPA concentration ($\mu$g/L), P is the observed BPA concentration ($\mu$g/L) and SG is the specific gravity of the urine sample (Boeniger *et al.*, 1993; Teass *et al.*, 1993). SG was measured using a handheld refractometer (National Instrument Company Inc., Baltimore, MD, USA), which was calibrated with deionized water before each measurement.

### Statistical analyses

Descriptive statistics and distributions of urinary BPA concentrations were tabulated and compared among demographic categories. Spearman correlation coefficients were calculated for total number of oocytes retrieved per cycle with peak serum oestradiol concentration and for SG-adjusted cycle specific urinary BPA concentration with BMI. The geometric mean of the two SG-adjusted urinary BPA concentrations collected during each IVF cycle was used as the cycle-specific urinary BPA concentration to reduce within-cycle variability, and was log-transformed in statistical analyses to reduce skewness. Mixed effect models were used to evaluate the association of the log SG-adjusted cycle-specific urinary BPA concentration with peak serum oestradiol concentration. Poisson regression models using a generalized estimating equation (GEE) approach were used to evaluate the association of the urinary BPA concentration with total number of oocytes retrieved. Both the mixed effect and Poisson regression models using autoregressive 1 (AR1) correlation structure accounted for correlation between repeated IVF cycles in the same woman, and adjusted for selected covariates which could be confounders. All statistical analyses were conducted using SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA). *p*-values <0.05 were considered statistically significant.

## Results

Urinary BPA concentrations were measured on 84 women who underwent IVF. The women ranged in age from 21 to 44 years, with a mean ± SD of 35.6 ± 3.9 (Table 1). Seventy-four (88%) women were Caucasian. BMI ranged from 16.5 to 42 kg/m$^2$, with a mean ± SD of 24.0 ± 5.1. Sixty (71%) women had never smoked and three women were current smokers. A majority of the subjects had SART diagnoses of female factor (35%) or male factor infertility (36%); unexplained infertility was present in 29% of the subjects. Day 3 FSH levels ranged from 1.0 to 15.2 IU/L, with a mean ± SD of 7.7 ± 2.3. These values were consistent with those seen in most IVF populations, as day 3 FSH levels greater than 15 IU/L are associated with decreased ovarian response including decreased peak oestradiol levels, number of oocytes retrieved and pregnancy rates (Scott *et al.*, 1989).

The 84 subjects contributed 112 IVF cycles, including 23 (27%) women who contributed more than one IVF cycle during the study period. Eighteen (21%) women contributed two IVF cycles and five (6%) women contributed three IVF cycles. Of the 112 IVF cycles, 74 (66%) cycles were luteal phase protocol cycles and 66 of these cycles utilized low-dose Lupron (Table 2). Peak oestradiol concentrations ranged from 551 to 4455 pg/mL, with a mean ± SD of 2004 ± 838. The total number of oocytes retrieved per IVF cycle ranged from 1 to 27, with a mean ± SD of 10.4 ± 5.3. Peak serum oestradiol levels showed a strong positive correlation with the total number of oocytes retrieved per cycle ($r = 0.65$, $p < 0.001$).

Urinary BPA concentrations were measured in 203 urine samples collected during 112 IVF cycles. Among the 203 urine samples, 31 (15%) had BPA concentrations below the LOD (0.4 $\mu$g/L). The median SG-adjusted BPA concentration was 2.61 $\mu$g/L (25th percentile,

1.44; 75th percentile, 4.32) with a geometric mean ± SD of 3.97 ± 5.9 (Table 3). SG-adjusted BPA concentrations ranged from <LOD to 65.3 $\mu$g/L. Urinary concentrations unadjusted for SG (Table 3) are also shown to allow comparisons with other studies. The distribution was similar to the previous studies (Calafat *et al.*, 2008; Mahalingaiah *et al.*, 2008), with a median of 1.60 $\mu$g/L (25th percentile, 0.70; 75th percentile 3.10).

Of the 112 cycles, 91 cycles contributed two urine samples and 21 cycles contributed only one urine sample. The cycle specific geometric median SG-adjusted BPA concentrations ranged from <LOD to 29.6 $\mu$g/L, with a median of 2.28 $\mu$g/L (25th percentile, 1.46; 75th percentile, 4.00) (Table 4). Of the 112 cycle-specific geometric mean urinary BPA concentrations, five (4%) were <LOD (Table 4). There was no association between SG-adjusted cycle specific urinary BPA concentration and BMI ($r = -0.06$, $p = 0.61$).

There were statistically significant univariate associations of day 3 FSH concentrations with measures of peak serum oestradiol and the number of oocytes retrieved, measures of ovarian response to hyperstimulation. For each unit increase in day 3 FSH (IU/L), there was an average decrease of 9% (95% CI: 5,14%; $p = 0.0001$) in the number of oocytes retrieved and an average decrease in peak oestradiol of 116 pg/mL (95% CI: −187,−45; $p = 0.002$). Age was not associated with decreased ovarian response.

Using a Poisson regression model with a GEE approach to account for multiple cycles in the same woman, and controlling for age, BMI and day 3 FSH level, there was an average decrease of 12% (95% CI: 4,23%; $p = 0.007$) in the number of oocytes retrieved per cycle for each log unit increase in cycle specific SG-BPA (Table 5). In addition, in a mixed effect regression model accounting for multiple IVF cycles, and adjusting for age, BMI and day 3 FSH level, there was an average decrease of 213 pg/ml (95% CI: −407, −20; $p = 0.03$) in peak serum oestradiol levels for each log unit increase in SG-BPA (Table 6).

## Discussion

In this study, urinary BPA concentrations were inversely associated with number of oocytes retrieved per cycle, with an average decrease of 12% for each log unit increase in SG-BPA. The number of oocytes retrieved was strongly correlated with peak serum oestradiol concentrations. This has been previously reported (Kossoy *et al.*, 1989; Loumaye *et al.*, 1997) and was expected because oestradiol plays a key role in oogenesis (Hewitt *et al.*, 2005). We also found an inverse association between urinary BPA concentrations and peak serum oestradiol levels. The decrease in peak oestradiol was consistent with our findings on the number of retrieved oocytes and our hypothesis of the adverse effects of BPA on overall ovarian response to controlled ovarian hyperstimulation.

Poor ovarian response to hyperstimulation occurs frequently and has been found to complicate an estimated 5–24% of all IVF cycles (Keay *et al.*, 1997). Although there is variation in the definition of poor responders, the association between poor ovarian response and significantly lower IVF success rates is well-known (Pellicer *et al.*, 1987; Jenkins *et al.*, 1991). While most of the earlier studies did not control for age, Saldeen *et al.* (2007), who defined poor ovarian response as ≤5 follicles at time of oocyte retrieval, found that poor responders had lower pregnancy rates compared with normal responders regardless of age (3 vs. 22%, $p < 0.05$ in women >37 years; 14 vs. 35%, $p < 0.05$ in women ≤37 years). They also found significantly higher IVF cycle cancellation rates in poor responders in both age groups (44 vs. 13%, 40 vs. 11%, $p < 0.05$).

Several experimental studies have demonstrated effects of environmentally relevant concentrations of BPA on ovarian granulosa cells, known to play a key role in folliculogenesis and steroidogenesis (Erickson & Shimasaki, 2001; Nilsson & Skinner,

2001). Xu *et al.* (2002) showed that ovarian granulosa cells cultured with 100 pM BPA for 24–72 h had decreased viability and increased apoptosis in both a dose- and time-dependent relationship. Using Western blots and real-time quantitative PCR, they demonstrated that 100 $\mu$M BPA, when compared with the control conditions, increased the protein expression and mRNA levels of Bax (199 and 243% respectively) and decreased those of Bc12 (82 and 77% respectively). The balance of anti-apoptotic (for example Bc12) and pro-apoptotic proteins (Bax) is essential for determining cell death or survival. Granulosa cell apoptosis plays an important role in follicular atresia (Hughes & Gorospe, 1991; Yu *et al.*, 2004). Research has also shown that BPA may partially antagonize the effects of oestradiol on ER$\alpha$, expressed in granulosa cells (Hiroi *et al.*, 1999). These results suggest that BPA may antagonize the anti-apoptotic effect of endogenous oestrogens synthesized by granulosa cells.

Further evidence of the effect of BPA on ovarian granulosa cells was provided by Mlynarcikova *et al.* (2005), who demonstrated decreased FSH-induced oestradiol production by porcine ovarian granulosa cells after 72 h incubation with BPA in the range of $10^{-8}$ – $10^{-4}$ M. More recently, in rat ovarian granulosa cells cultured in vitro with BPA, Zhou *et al.* (2008) demonstrated a concentration-dependent inhibitory effect of BPA ($10^{-6}$–$10^{-4}$ M) on oestradiol levels and the expression of P450arom mRNA. The decrease in oestradiol production was shown to be mediated, in part, by disruption of ovarian steroidogenic enzymes, specifically the mRNA expression of P450arom, a key enzyme in oestradiol synthesis.

Bisphenol A has also been shown to have deleterious effects on meiotic chromosome segregation in experimental animals. The aetiology of the effects of BPA on oocyte number is likely through the disruption of oogenesis and induction of meiotic aneuploidy as demonstrated in experimental animals (Hunt *et al.*, 2003; Can *et al.*, 2005; Susiarjo *et al.*, 2007; Lenie *et al.*, 2008). Hunt *et al.* (2003) initially observed a large increase in congression failure in oocytes from young control mice from the usual 1–2% to up to 40% of oocytes. This observation coincided with an accidental exposure of the mice to BPA from damaged polycarbonate cages and water bottles. They performed subsequent experiments to confirm these findings, in which young mice were intentionally exposed to oral BPA doses of 20–100 ng/g body weight/day for 7 days, and found a significant dose-related increase in per cent of oocytes with congression failure (1.7% in the control group, 5.8% at 20 ng/g bw/day, 7.5% at 40 ng/g bw/day and 10.9% at 100 ng/g bw/day).

Results from other recent experiments have suggested that the effect of BPA on mammalian oogenesis occurs as early as the onset of meiosis in the foetal ovary. Susiarjo *et al.* (2007) treated pregnant mice with 20 $\mu$g/kg bw/day of BPA for 7 days through implanted time-release pellets and reported an increase in synaptic abnormalities and increased levels of recombination in oocytes isolated from exposed female foetuses during the mid-gestation period. Another subgroup of treated mice were allowed to carry to term and oocytes that were subsequently obtained from the adult female offspring were found to have an increased level of aneuploidy, from 1.8% of the oocytes in the placebo group to 21.4% in the BPA-treated group ($p < 0.001$). The researchers also observed the same meiotic defects in foetal ovaries of mice homozygous for a targeted disruption of ER$\beta$ and found no additional effects of BPA exposure in ER$\beta$ null females. These findings suggest that BPA may disrupt early oogenesis by interfering with the actions of ER$\beta$, one of the two known oestrogen receptors.

Human studies on potential reproductive effects of BPA are rather limited and as far as we are aware, we report some of the first data on the association of urinary BPA concentrations with ovarian response. Current literature includes a retrospective study that found higher

mean serum BPA levels in 45 Japanese women with recurrent miscarriages compared with healthy, nulligravid women (Sugiura-Ogasawara *et al.*, 2005). However, this study had several limitations including a small sample size, retrospective assessment of serum bisphenol A concentrations measured after the recurrent miscarriages, and use of ELISA to measure BPA, which is a less specific method compared with HPLC (Fukata *et al.*, 2006). A more recent study investigated serum BPA concentrations and IVF outcomes in 44 women and found an inverse association between serum BPA and oestradiol levels ($p = 0.06$), as well as a trend of higher serum BPA levels in women who did not achieve pregnancy compared with those who did ($p = 0.57$) (Lamb *et al.*, 2008). These results are consistent with our findings in this study on the association between BPA and reduced ovarian response in women undergoing IVF.

Bisphenol A concentrations were detectable in the majority of the urine samples in this study, which is consistent with the NHANES 2003–2004 data, confirming widespread exposure to BPA (Calafat *et al.*, 2008). The urinary concentrations found in this study were also similar in magnitude to NHANES 2003–2004, which reported a geometric mean (non-SG adjusted) BPA concentration of 2.6 $\mu$g/L among men and women between 20 and 59 years of age. In this study the geometric mean (non-SG adjusted) urinary BPA concentration was 2.52 $\mu$g/L.

To the best of our knowledge, this study is the first environmental reproductive epidemiological study to use multivariable mixed effect and Poisson regression models to account for correlation between multiple IVF cycles within the same woman. Most studies in the literature circumvent the issue of analysing repeated measures data from the same subject by including only the first IVF cycle. Although a valid approach, this leads to low power as a large portion of the data is excluded from the statistical analysis. Therefore, the statistical methods utilized in this study allow for a greater power to detect associations between environmental exposures and fertility outcomes, as many women receiving infertility treatment often undergo more than one IVF cycle.

One potential limitation of this study is uncertainty regarding the generalizability of findings to women who are not receiving infertility treatment. The IVF population was chosen as it allowed for assessment of early developmental endpoints that are otherwise not observable in women conceiving naturally, at stages that are likely to be the most sensitive to BPA as shown in experimental animal studies. Another limitation is the small sample size, which limits power and the ability to perform stratum-specific analyses within SART diagnoses. The present results should be considered preliminary as the study is ongoing. Our statistical models were adjusted for age, BMI and Day 3 FSH levels as increased age and BMI and decreased ovarian reserve have been identified in other studies as independently associated with decreased ovarian response in women undergoing IVF. Finally, it is unclear whether the number and timing of urinary collections allow for an accurate measurement of BPA exposure. BPA has a short half-life and its concentration in a spot urine sample is only reflective of recent exposure. Although a single sample has been shown to be predictive of long-term exposure of weeks or months (Mahalingaiah *et al.*, 2008) and having two urinary measurements is superior to one, it is unclear whether these measurements can adequately categorize BPA exposure over the course of the IVF cycle. Furthermore, the relevant timing of BPA exposure in relation to follicle development is unknown. It is possible that the exposure should be measured months, or even years, prior to oocyte retrieval to assess the effects on oogenesis.

## Conclusion

Bisphenol A was detected in the majority of women undergoing IVF, and BPA urinary concentrations were found to be inversely associated with the number of oocytes retrieved per cycle and peak serum oestradiol levels. Further work on the association of urinary BPA concentrations with additional IVF endpoints is underway, including embryo quality and implantation rate, as well as effects on the developing foetus. Ultimately, the association of urinary BPA concentrations with term pregnancy and live birth rates is of greatest importance to the infertile population. However, the possibility of long-term effects on reproductive health for all women also warrants research, including potential consequences of in-utero exposure of female foetuses to BPA on subsequent adult ovarian function.

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## Table 1

Subject demographics, fertility diagnoses and day 3 FSH concentrations

| Characteristic | Subjects (*n* = 84) |
|---|---|
| Age (years) | |
| Mean ± SD | 35.6 ± 3.9 |
| Range | 21–44 |
| BMI (kg/m$^2$) | |
| Mean ± SD | 24.0 ± 5.1 |
| Range | 16.5–42 |
| Race [*n*(%)] | |
| Caucasian | 74 (88) |
| African-American/Black | 3 (4) |
| Hispanic | 4 (5) |
| Other | 3 (4) |
| Smoking history [*n*(%)] | |
| Never | 60 (71) |
| Former | 21 (25) |
| Current | 3 (4) |
| SART diagnosis [*n*(%)] | |
| Female factor | 29 (35) |
| Endometriosis | 8 (10) |
| Tubal factor | 7 (8) |
| Diminished ovarian reserve | 7 (8) |
| Ovulation disorders | 6 (7) |
| Uterine disorders | 1 (1) |
| Male factor | 30 (36) |
| Unexplained | 24 (29) |
| Other | 1 (1) |
| Day 3 FSH (IU/L) | |
| Mean ± SD | 7.7 ± 2.3 |
| Range | 1–15.2 |

BMI, body mass index; SART, Society for Assisted Reproductive Technology; FSH, follicle-stimulating hormone.

## Table 2

Treatment protocols and ovarian response for 112 IVF cycles among 84 women

| | **Cycles (*n* = 112)** |
|---|---|
| IVF protocol [*n*(%)] | |
| Luteal phase | 74 (66) |
| LDLL | 66 (59) |
| RDLL | 7 (6) |
| HDLL | 1 (1) |
| Flare | 26 (23) |
| Antagonist | 12 (11) |
| hCG trigger dose [*n*(%)] | |
| ≤5000 IU | 24 (21) |
| 10 000 IU | 88 (79) |
| Peak oestradiol (pg/ml) | |
| Mean ± SD | 2004 ± 838 |
| Range | 551–4455 |
| Total oocytes retrieved per cycle (*n*) | |
| Mean ± SD | 10.4 ± 5.3 |
| Range | 1–27 |

Eighteen women contributed two IVF cycles, five women contributed three IVF cycles.

IVF, in vitro fertilization; LDLL, low-dose leuprolide lupron; RDLL, regular-dose leuprolide lupron; HDLL, high-dose leuprolide lupron; hCG, human chorionic gonadotropin.

**Table 3**

Distribution of 203 urinary BPA concentrations ($\mu$g/L) in 112 IVF cycles from 84 subjects

| | Geomean (SD) | Min | 10th | 25th | 50th | 75th | 90th | 95th | Max |
|---|---|---|---|---|---|---|---|---|---|
| BPA | 2.52 (3.2) | 0.18 | 0.20 | 0.70 | 1.60 | 3.10 | 5.00 | 8.70 | 25.5 |
| SG-adjusted BPA | 3.97 (5.9) | 0.18 | 0.86 | 1.44 | 2.61 | 4.32 | 8.16 | 11.0 | 65.3 |

Twenty-one of 224 (9%) urine samples were missing, such that 91 cycles contributed two urine samples and 21 cycles contributed one urine sample. Thirty-one (15%) urine samples were below LOD (<0.4 $\mu$g/L) and are included in the percentiles as LOD/2.

BPA, bisphenol A; SG, specific gravity; Geomean, geometric mean.

**Table 4**

Distribution of cycle-specific urinary BPA concentrations ($\mu$g/L) in 112 cycles from 84 subjects (geometric mean)

| | Geomean (SD) | Min | 10th | 25th | 50th | 75th | 90th | 95th | Max |
|---|---|---|---|---|---|---|---|---|---|
| BPA | 2.11 (2.6) | 0.18 | 0.44 | 0.70 | 1.45 | 2.92 | 3.87 | 5.16 | 22.1 |
| SG-adjusted BPA | 3.27 (3.3) | 0.18 | 1.12 | 1.46 | 2.28 | 4.00 | 6.00 | 7.79 | 29.6 |

Five (4%) cycle-specific BPA concentrations were below LOD (<0.4 $\mu$g/L) and included in the percentiles as LOD/2.

BPA, bisphenol A; SG, specific gravity; Geomean, geometic mean.

## Table 5

Poisson model for the association of urinary BPA concentrations with number of oocytes retrieved, after adjusting for age, BMI and day 3 FSH levels and accounting for multiple IVF cycles

| Variable | Incidence Rate Ratio | 95% Confidence Interval | *p*-value |
|---|---|---|---|
| Ln(SG-BPA) | 0.88 | (0.77, 0.96) | 0.007 |
| Age | 0.99 | (0.96, 1.01) | 0.34 |
| BMI | 1.00 | (0.98, 1.02) | 0.92 |
| Day 3 FSH | 0.91 | (0.86, 0.95) | <0.0001 |

Ln, natural logarithm; SG-BPA, specific gravity-adjusted bisphenol A; FSH, follicle-stimulating hormone; IVF, in vitro fertilization; BMI, body mass index.

## Table 6

Mixed effect model for the association of urinary BPA concentrations with peak oestradiol level, after adjusting for age, BMI and day 3 FSH levels and accounting for multiple IVF cycles

| Variable | Parameter estimate | 95% Confidence interval | *p*-value |
|---|---|---|---|
| Ln(SG-BPA) | −213 | (−407, −20) | 0.03 |
| Age | −7 | (−45, 31) | 0.71 |
| BMI | −20 | (−50, 9) | 0.17 |
| Day 3 FSH | −120 | (−185, −55) | 0.0004 |

Ln, natural logarithm; SG-BPA, specific gravity-adjusted bisphenol A; FSH, follicle-stimulating hormone; IVF, in vitro fertilization; BMI, body mass index.