Urine bisphenol-A (BPA) level in relation to semen quality

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Objective: To determine whether urine bisphenol-A (BPA) levels are associated with lower semen quality.
Design: Cohort study.
Setting: Four regions in China where high exposure to BPA in the workplace existed.
Patient(s): 218 men with and without BPA exposure in the workplace.
Intervention(s): None.
Main Outcome Measure(s): Semen parameters.
Result(s): After adjustment for potential confounders using linear regression, increasing urine BPA level was statistically significantly associated with [1] decreased sperm concentration, [2] decreased total sperm count, [3] decreased sperm vitality, and [4] decreased sperm motility. Compared with men who did not have detectable urine BPA levels, those with detectable urine BPA had more than three times the risk of lowered sperm concentration and lower sperm vitality, more than four times the risk of lower sperm count, and more than twice the risk of lower sperm motility. The urine BPA level was not associated with semen volume or abnormal sperm morphology. Similar dose-response associations were observed among men with environmental BPA exposure at levels comparable with those in the U.S population. Despite a markedly reduced sample size, the inverse correlation between increased urine BPA levels and decreased sperm concentration and total sperm count remained statistically significant.
Conclusion(s): These results provide the first epidemiologic evidence of an adverse effect of BPA on semen quality. (Fertil Steril® 2011;95:625–30. ©2011 by American Society for Reproductive Medicine.)

Key Words: Bisphenol A, BPA, endocrine disruptor, epidemiology, male fertility, semen quality

Bisphenol-A (BPA) is a suspected potent endocrine disruptor, with endocrine-disrupting properties demonstrated in animal studies (1). If these properties are confirmed in human epidemiologic studies, BPA poses a significant public health hazard because of its widespread presence in the environment and consumer products. Bisphenol-A is now contained in a wide variety of consumer products, from baby bottles, plastic containers, and the resin lining of cans for food and beverages to dental sealants (1). Humans have been shown to have increased urine BPA levels after using these products (2), and BPA can be detected in a majority of the U.S. population and populations of other countries (3–6).

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The endocrine-disrupting properties of BPA have largely been demonstrated via in vitro and in vivo experimental animal studies, where BPA has exhibited both estrogenic and antiandrogenic effects (1, 7–12). Animal studies have shown that BPA affects the male reproductive organs, including the testes, epididymis, sperm and seminal vesicles, prostate gland, and sperm production (1, 7, 13–18). Bisphenol-A also interferes with the function of androgen receptors and the production of male sex hormones (7, 8, 19). However, human studies demonstrating BPA's endocrine-disrupting effects have been sparse (19, 20). We recently reported a high risk of male sexual dysfunction associated with exposure to BPA (21, 22), providing the first piece of evidence of BPA's endocrine-disruptive effect in human populations. Nevertheless, additional human studies to examine the detrimental effects of BPA that have been reported in animal studies are urgently needed to determine BPA's safety. Our current study was designed to evaluate the relationship between urine BPA level, a biological marker of BPA exposure, and semen quality, an objective measure of damage to the male reproductive system, to which animal studies have previously demonstrated BPA's adverse impact.

MATERIALS AND METHODS

See the Supplemental Materials online for the full description. A detailed description of our study can be found elsewhere (21, 22). The following are brief descriptions of the study population and relevant methods.

Study Population

Workers in participating factories with and without BPA exposure in the workplace were identified as eligible for our study. Potential participants were not aware of the specific hypotheses of the study. Among 888 eligible workers, 514 (58%) agreed to participate in the study. All participants were asked to provide both urine and semen specimens. Through an in-person interview, participants provided information on demographic characteristics and occupational history; on potential risk factors that might influence semen quality, including smoking, alcohol use, chronic diseases, history of subfertility, and exposure to other chemicals and heavy metals; and on recent exposure to heat sources such as a steam bath.

BPA Measurement

For each participating worker with BPA exposure in the workplace, two spot urine samples, preshift and postshift, were collected. For workers without BPA exposure in the workplace, one urine specimen was collected. For each urine sample, the total urine BPA concentration (free plus conjugated species) was measured using high-performance liquid chromatography (HPLC) as described by He et al. (23). The limit of detection (LOD) was 0.31 μg/L, which is comparable to that reported by previous studies (3, 24).

The urine BPA level was measured by both volume-based (micrograms per liter) and creatinine-corrected (micrograms per gram creatinine) concentrations. To better represent the actual BPA exposure levels for those who had BPA exposure in the workplace, we averaged the BPA concentrations of the preshift and postshift samples.

Semen Analysis

All participants were asked to provide two semen specimens for analysis, with the interval between the two samples ranging from 7 to 21 days. Collection and examination of the semen specimens strictly followed the World Health Organization (WHO) standards and requirements (25). We restricted our analyses to participants who met the sexual abstinence requirement (2 to 7 days), and the semen samples were analyzed within 1 hour of ejaculation, according to the WHO semen collection guidelines.

Semen specimens were carefully prepared according to WHO instructions (25). To be consistent, the semen analysis for all specimens was conducted by the same technician. In addition to the manual examination, we used Computer Assisted Sperm Analysis (CASA) (WLJY-9000, Beijing, PRC) to examine all the parameters of semen quality except for sperm morphology, for which CASA has not been considered reliable.

We examined the association between BPA urine levels and semen quality using six common semen quality parameters: volume, total sperm count, concentration, vitality, motility (forward movement [grades A + B]), and morphology. Due to a lack of variation for pH level in this study population, we were not able to use pH level as one of the end points to be examined.

Analyses

The parameters measuring semen quality in the analyses were all continuous variables. We first used general linear regression to examine the correlation between the urine BPA level (a continuous variable) and various parameters of semen quality after adjustment for potential confounders. Because of the skewed distribution of urine BPA data, we included the variable in the regression model after $\log_{10}$-transformation. Before the $\log_{10}$-transformation, urine BPA values of 0 were inputed as LOD/($\sqrt{2}$), based on conventionally accepted practice (26).

To make the results more interpretable, we also measured the association using odds ratios (OR) based on dichotomized outcomes. We dichotomized the parameters based on the median value of each parameter. We used logistic regression and its confidence interval (CI) to examine the association between urine BPA levels and various parameters of lower semen quality after adjustment for potential confounders.

Supplemental Figure 1 (available online) describes recruitment and the criteria for inclusion and exclusion. Ultimately, 218 men who provided both urine BPA and semen specimens were included in the final analyses.

RESULTS

The distribution of BPA levels by the characteristics of the study population is presented in Supplemental Table 1 (available online). Participants with more advanced education and longer employment history had relatively lower BPA levels. The most important determining factor for a high BPA level in this study population was exposure to BPA in the workplace. There was no clear pattern of BPA distribution by age. The urine BPA level was not associated with marital status, a history of chronic diseases that may impact semen quality, alcohol intake, smoking, exposure to other chemicals or heavy metals, or a history of subfertility. Only a small number of participants were exposed to sauna/steam baths in this population; thus, the relationship of this heat factor with BPA level was difficult to assess.

Supplemental Table 2 (available online) presents the distribution of the semen parameters in the study population. Although no comparable data for semen quality were well documented in the populations of the study regions, the parameters of semen quality in Supplemental Table 2 were comparable with the parameters among the Chinese population published in other studies (27, 28).

After adjustment by linear regression for age, education, history of chronic diseases, previous exposure to other chemicals or heavy metals, employment history, marital status, age at first intercourse, smoking and alcohol drinking status, and study site, we observed a highly statistically significant linear correlation (a linear dose-response) between increasing urine BPA concentration and declining semen quality as measured by concentration, total sperm count, vitality, and motility (Table 1). Specifically, an increasing urine BPA level was associated with lower semen concentration ($P<.001$), lower total sperm count ($P=.004$), lower sperm vitality ($P<.001$), and lower sperm motility (forward movement) ($P<.001$). There was no observed linear correlation between urine BPA and semen volume or sperm morphology.

To measure the association between urine BPA level and lower semen quality in terms of relative risk for lower semen quality (lower than median level of semen parameters), we also obtained

TABLE 1

Linear correlation between urine bisphenol-A (BPA) level[a] and measurements of semen quality.

Semen parameter	N	Adjusted[b] β coeffcient	*P* value
Concentration ($\times 10^6$ sperm/mL)	215	–15.6	<.001
Total sperm count ($\times 10^6$)	215	–42.1	.004
Sperm vitality (% alive)	217	–4.6	<.001
Sperm motility (% moving forward)[c]	218	–3.1	<.001
Semen volume (mL)	218	0.1	.40
Sperm morphology[d] (% normal)	217	0.05	.95

[a] μg/gCr, $\log_{10}$-transformed.
[b] Adjusted for age, education, history of chronic disease, previous exposure to other chemicals and heavy metals, employment history, marital status, age at first intercourse, smoking, drinking, and study site.
[c] A + B movement: Rapid (A) + slow (B) progressive (forward) movement.
[d] Additionally adjusted for occupational exposure to BPA due to significant improvement in model fit.

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TABLE 2

Urine bisphenol-A (BPA) level[a] and semen quality.

Semen parameter	BPA detected	Semen parameter level: ≤ Median, N (%)	Semen parameter level: > Median, N (%)	aOR[a,b] (95% CI) for ≤ Median
Concentration ($\times 10^6$ sperm/mL)	No	17 (32.7)	35 (67.3)	Reference
	Yes	91 (55.8)	72 (44.2)	3.4 (1.4–7.9)
Total sperm count ($\times 10^6$)	No	17 (32.7)	35 (67.3)	Reference
	Yes	92 (56.4)	71 (43.6)	4.1 (1.7–9.9)
Sperm vitality (% alive)	No	23 (43.4)	30 (56.6)	Reference
	Yes	87 (53.1)	77 (47.0)	3.3 (1.4–7.5)
Sperm motility[c] (% moving forward)	No	26 (49.1)	27 (50.9)	Reference
	Yes	89 (53.9)	76 (46.1)	2.3 (1.0–5.1)
Semen volume (mL)	No	26 (49.1)	27 (50.9)	Reference
	Yes	85 (51.5)	80 (48.5)	1.2 (0.5–2.6)
Sperm morphology[d] (% normal)	No	35 (66.0)	18 (34.0)	Reference
	Yes	75 (45.7)	89 (54.3)	0.7 (0.3–1.6)

[a] μg/gCr.
[b] Adjusted odds ratio for age, education, history of chronic disease, previous exposure to other chemicals and heavy metals, employment history, marital status, age at first intercourse, smoking, drinking, and study site.
[c] A + B movement: Rapid (A) + slow (B) progressive (forward) movement.
[d] Additionally adjusted for occupational exposure to BPA, due to significant improvement in model fit.

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odds ratios using logistic regression to control for confounders. We did not use the WHO criteria of abnormality used mainly for identifying infertility because the number of participants who met the WHO criteria was too small for the analysis. Compared with the men who had no detectable urine BPA, those with detectable urine BPA had more than three times the risk of having lower sperm concentration (lower than median level) (adjusted OR [aOR] = 3.4; 95% CI, 1.4–7.9) and lower sperm vitality (aOR = 3.3; 95% CI, 1.4–7.5), more than four times the risk of lower sperm count (aOR = 4.1; 95% CI: 1.7–9.9), and more than twice the risk of lower sperm motility (aOR = 2.3; 95% CI, 1.0–5.1). Again, the urine BPA level was not associated with semen volume or abnormal sperm morphology (Table 2).

To further examine whether the observed association demonstrated a dose-response relationship between increasing urine BPA concentration and lower semen quality (lower than median value), we divided the men with detectable urine BPA levels into three categories of tertiles. As the results in Table 3 show, increasing urine BPA levels according to the three tertile levels were generally associated with a greater risk of lower semen quality for parameters measuring sperm concentration, vitality, and motility, confirming the results of linear regression analysis presented in Table 1.

To determine whether the observed association also existed among participants with only environmental BPA exposure (generally a lower BPA exposure level compared with those who had occupational BPA exposure), we examined the association after

TABLE 3

Dose-response relationship between urine bisphenol-A (BPA) level[a] and lower semen quality.

Semen parameter	N	aOR[b] (95% CI) for ≤ median on semen parameter: BPA undetectable	Lowest tertile BPA	Middle tertile BPA	Highest tertile BPA
Concentration ($\times 10^6$ sperm/mL)	215	Reference	2.3 (0.9–6.2)	3.6 (1.3–9.6)	4.7 (1.7–13.6)
Total sperm count ($\times 10^6$)	215	Reference	4.2 (1.5–11.3)	4.4 (1.6–11.8)	3.8 (1.3–10.7)
Sperm vitality (% alive)	217	Reference	1.8 (0.7–4.7)	4.0 (1.5–10.4)	5.9 (2.0–17.2)
Sperm motility[c] (% moving forward)	218	Reference	1.6 (0.6–4.1)	2.4 (0.9–5.9)	3.6 (1.3–9.9)
Semen volume (mL)	218	Reference	1.6 (0.6–4.0)	1.3 (0.5–3.2)	0.8 (0.3–2.0)
Sperm morphology[d] (% normal)	217	Reference	0.7 (0.3–2.0)	0.7 (0.3–1.9)	0.6 (0.2–1.7)

[a] μg/gCr.
[b] Adjusted odds ratio for age, education, history of chronic disease, previous exposure to other chemicals and heavy metals, employment history, marital status, age at first intercourse, smoking, drinking, and study site.
[c] A + B movement: Rapid (A) + slow (B) progressive (forward) movement.
[d] Additionally adjusted for occupational exposure to BPA, due to significant improvement in model fit.

Li. Urine BPA level and semen quality. Fertil Steril 2011.

TABLE 4

Linear correlation between urine bisphenol-A (BPA) level[a] and measurements of semen quality among those exposed to environmental BPA sources only.

Semen parameter	N	Adjusted[b] β coeffcient	*P* value
Concentration ($\times 10^6$ sperm/mL)	87	–22.3	.02
Total sperm count ($\times 10^6$)	87	–79.0	.04
Sperm vitality (% alive)	88	–1.5	.49
Sperm motility (% moving forward)[c]	88	–1.3	.49
Semen volume (mL)	88	–0.2	.40
Sperm morphology (% normal)	88	–0.9	.54

[a] μg/gCr, $\log_{10}$-transformed.
[b] Adjusted for age, education, history of chronic disease, previous exposure to other chemicals and heavy metals, employment history, marital status, age at first intercourse, smoking, drinking, and study site.
[c] A + B movement: Rapid (A) + slow (B) progressive (forward) movement.

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excluding the men who had BPA exposure in the workplace. Similar associations of urine BPA level with semen concentration, total sperm count, and sperm vitality were also observed among the men with only environmental BPA exposure, whose average BPA exposure level was slightly lower than the BPA level detected in the general U.S. population (3). Despite a markedly reduced sample size, the inverse correlation between increased urine BPA level and decreased sperm concentration and total sperm counts remained statistically significant (Table 4).

Although creatinine-corrected BPA concentration was used in the analysis, using the original volume-based concentration without correction for creatinine level produced similar results. The previous analyses were based on the semen parameters determined by manual examination, but using semen parameters determined by the CASA method produced essentially the same results. The consistency of the results between manual and CASA methods of semen analyses provides assurance of the quality of semen analyses.

Five participants had varicocele recorded during physical examination. Excluding those five men from analyses did not change the results.

DISCUSSION

Animal studies have shown that exposure to BPA could have detrimental effects on the male reproductive system (1, 7–13, 29). As a potent endocrine disruptor, BPA has been shown to have both estrogenic and antiandrogenic properties, which provides biological plausibility for an adverse effect of BPA on the male reproductive system. However, human studies examining this effect have been limited, and more studies are only now emerging. We recently reported on an increased risk of male sexual dysfunction associated with BPA exposure (21, 22), and others have reported a BPA effect on the cardiovascular system (30) and hormone levels (31).

The results from our present study provide the first piece of epidemiologic evidence that exposure to high BPA levels has an adverse effect on semen quality in an adult human population. Not only was having a detectable urine BPA level associated with an increased risk of lower semen quality, but the association demonstrated a dose-response relationship with a highly statistically significant linear trend (Tables 1 to 4). The inverse relationship between increasing urine BPA level and decreased semen quality was observed even among those with low BPA exposure—that is, lower than the BPA level in the U.S. population (3)—from environmental sources. The four parameters measuring semen quality (sperm concentration, total sperm count, vitality, and motility) that were associated with the urine BPA level observed among our study population were also found to be associated with BPA exposure in animal studies (1, 7, 32). This consistency between human and animal study findings further supports an underlying association.

The mechanisms of the reported adverse effect of BPA on semen quality (33–36) are not yet completely understood, but some studies have shown that BPA may have a direct adverse impact on spermatogenesis (34). Oxidative stress on sperm by BPA has also been proposed as a potential mechanism for its adverse effect (32). In addition, BPA acts as an androgen receptor (AR) antagonist that interrupts the normal AR binding activity and the interaction between AR and endogenous androgens (8, 13). Such an interruption by BPA on the function of endogenous androgens could conceivably interfere with normal spermatogenesis, which involves endogenous hormones. Also, BPA has been shown to impact the function of Leydig cells, resulting in a reduction of testosterone biosynthesis, which is important in sperm production and formation (37), and to affect several tissue and cell structures of male sexual organs through various mechanisms that include possible epigenetic effects (8). Finally, the estrogenic effect of BPA could potentially interfere with the hormonal balance, thus impacting spermatogenesis. Therefore, our observed inverse dose-response correlation between increasing urine BPA levels and declining semen quality is supported by biologic plausibility and the findings of experimental studies.

In addition to the strengths of our study (including a biological measurement of urine BPA level and semen specimens), some of the limitations of the study need to be kept in mind. Like many studies of this nature that, among other demands, require providing biologic specimens of both urine and semen, some of the study participants declined. We evaluated the potential impact of the nonparticipation. First, in order to have participation bias, nonparticipation had to be associated with semen quality and urine BPA level. As the eligible subjects were not likely to know their semen quality when they decided on participation in the study, it was unlikely that participation was associated with semen quality. Second, we examined whether the nonparticipation was associated with urine BPA level. We did not have information about urine BPA level for nonparticipants, so we examined the participation pattern between the workers with occupational BPA exposure, who usually had a high urine BPA level, and the workers without occupational BPA exposure, who usually had a low urine BPA level. Comparing age, educational level, and employment history (the only information available for nonparticipants) between the participants and nonparticipants, the participation pattern was quite similar among the men with high and low BPA exposures. Therefore, it seemed unlikely that the observed association between urine BPA level and lower semen quality could be explained by participation bias.

Even though we included workers with high BPA exposure (i.e., those with BPA exposure in the workplace), the median urine BPA level in this group (38.7 μg/L) remained almost 70 times below

the currently accepted tolerable daily intake and reference dose (2,687.5 μg/L, the urine level corresponding to the 0.05 mg/kg/day reference dose) set by the U.S. Environmental Protection Agency (2007) and the European Food Safety Authority (EFSA, 2007). In addition, we evaluated the association between urine BPA level and semen quality after excluding the men with high BPA exposure in the workplace. The remaining workers had BPA exposure only from environmental sources, and their median urine BPA level (1.4 μg/g creatinine-adjusted) was lower than that reported among the U.S. male population (median of 2.3 μg/g creatinine-adjusted) (3). A similar inverse association was observed among the participants with low environmental BPA exposure only (Table 4). Despite a markedly reduced sample size, the inverse correlation between increasing urine BPA levels and reduced sperm concentration and sperm counts remained statistically significant (Table 4).

CONCLUSION

This study presents for the first time evidence of an association between a BPA exposure biomarker (urine BPA level) and declining semen quality—specifically, reduced sperm concentration, total sperm count, vitality, and motility—in a human population. The association also demonstrated a dose-response relationship between increasing urine BPA level and reduction in semen quality among those with low environmental BPA exposure levels as well as those with high BPA exposure levels from the workplace. This finding has important implications for the public at large as well as for regulatory agencies. While this is the first time such an association has been reported in the human population, results from animal studies provide support for the underlying biological plausibility of the observed association. Evidence has started to accumulate that the adverse health effects of BPA observed in animal studies are being confirmed in epidemiologic studies.

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SUPPLEMENTAL MATERIALS AND METHODS

From 2004 to 2008, we conducted a study examining the health effect of BPA in several regions in China with high exposure to BPA in the workplace. Workers with and without exposure to BPA in the workplace were identified and recruited for the study. The occupational BPA exposure mainly came from epoxy resin manufacturers, who use BPA as one of their raw materials. Two Chinese academic and research institutions participated in data collection for the study. The committees for protection of human subjects from all participating institutes, including the institutional review board of Kaiser Permanente as well as the counterparts from the two Chinese collaborating institutions, approved the research and its protocols. We obtained informed consent from all participants before their participation in the study. A detailed description of the study protocol can be found elsewhere (21, 22). The following are brief descriptions of the study population and methods relevant to the present study.

Study Population

Workers in participating factories with and without BPA exposure in the workplace were identified as eligible for the present study. The original study was designed to examine the effect of occupational BPA exposure on the health of workers. Because unexposed workers also had BPA exposure from environmental sources and the occupationally exposed workers had BPA exposure at various levels, the current study examined the relationship between urine BPA levels and semen quality among all participating workers regardless of the source of their BPA exposure (occupational or environmental).

Exposed workers came from factories with confirmed BPA exposure in the workplace through both spot and personal air sampling measurements. The unexposed workers came from factories with no known BPA exposure in the workplace. Only male workers identified for this study were included in the present analysis. Potential participants were not aware of the specific hypotheses of the study. Among 888 eligible workers, 514 (58%) agreed to participate in the study. All participants were asked to provide both urine and semen specimens. Some participants were not included in this analysis due to a lack of semen specimens, urine specimens, or both. The main reasons for the lack of specimens were either refusal by participants or their being off-shift on the days of specimen collection. Through an in-person interview, participants provided information on demographic characteristics and occupational history; on potential risk factors that may influence semen quality, including smoking, alcohol use, chronic diseases, history of subfertility, or exposure to other chemicals and heavy metals; and on recent exposure to heat sources such as a steam bath.

BPA Measurement

For each participating worker with BPA exposure in the workplace, two spot urine samples, preshift and postshift, were collected. For workers without BPA exposure in the workplace, one urine specimen was collected. For each urine sample, the total urine BPA concentration (free plus conjugated species) was measured using high-performance liquid chromatography (HPLC) as described by He et al. (23). Briefly, urine samples were mixed with phosphorous acid buffer and β-glucuronidase (Sigma Chemical Co., St. Louis, MO) for hydrolyzation. Samples were then extracted twice with ether (HPLC grade; Dikma Ltd., Beijing, PRC), and the supernatants were evaporated with nitrogen gas. The residue was dissolved in 60% acetonitrile and analyzed by HPLC. The limit of detection (LOD) was 0.31 μg/L, which is comparable that reported by previous studies (3, 24).

The urine BPA level was measured by both volume-based (micrograms per liter) and creatinine-corrected (micrograms per gram creatinine) concentrations. To adjust for urine volume, we used a creatinine-corrected (μg/g Cr) BPA concentration in the analyses. To better represent the actual BPA exposure levels for those who had BPA exposure in the workplace, we averaged the BPA concentrations of the preshift and postshift samples. Thirty-two participants who did not provide urine samples for the BPA assay were excluded from the analysis.

Semen Analysis

All participants were asked to provide two semen specimens for analysis with the interval between the two samples ranging from 7-21 days. Collection and examination of semen specimens strictly followed the World Health Organization (WHO) standards and requirements (25). Participants were told to abstain from sexual activity for at least 2 days (but not exceeding 7 days) before the specimen collection. We restricted our analyses to the participants who met the sexual abstinence requirement, and the semen samples were analyzed within 1 hour of ejaculation, according to the WHO semen collection guidelines.

Following the WHO standards, participants were situated in a clean, private room with a constant temperature of 20° to 28°C. Semen specimens were obtained through masturbation and ejaculation into a clean, wide-mouthed container. Semen specimens were carefully prepared according to WHO instructions. Both macroscopic examination (e.g., liquefaction, appearance, viscosity, volume, and pH) and microscopic analysis (e.g., concentration, motility, vitality, and morphology) were performed according to the WHO manual (25). To be consistent, semen analysis for all specimens was conducted by the same technician. In addition to the manual examination, we used Computer Assisted Sperm Analysis (CASA) (WLJY-9000, Beijing, PRC) to examine all the parameters of semen quality except for sperm morphology, for which CASA has not been considered reliable.

We examined the association between BPA urine levels and semen quality using six common semen quality parameters: volume, total sperm count, concentration, vitality, motility (forward movement [grades A + B]), and morphology. Due to a lack of variation for pH level in this study population, we were not able to use pH level as one of the end points to be examined.

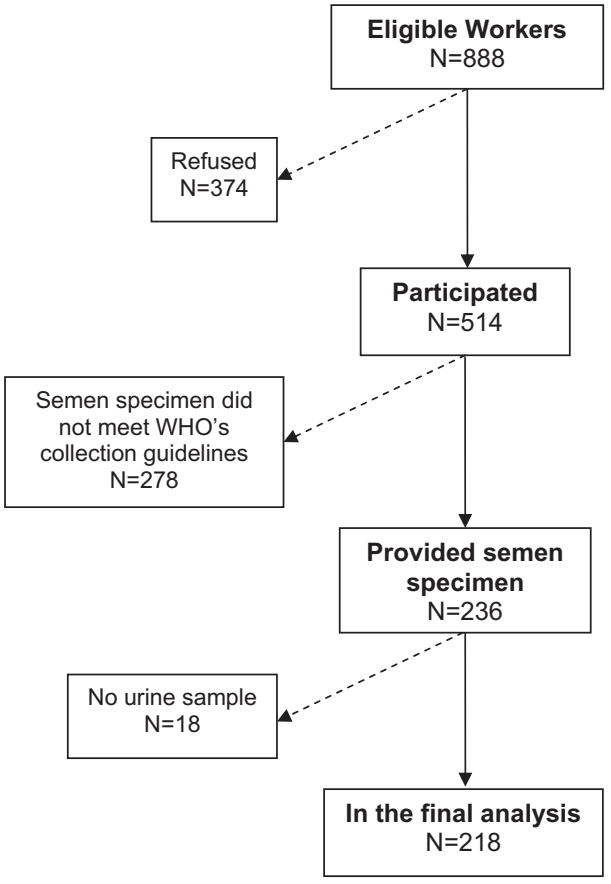
Analyses

The parameters measuring semen quality in the analyses were all continuous variables. We first used general linear regression to examine the correlation between the urine BPA level (a continuous variable) and various parameters of semen quality after adjustment for potential confounders. A statistically significant correlation could provide information on [1] the existence of an association, [2] the direction of the association (positive or inverse association), and [3] a potential linear dose-response relationship. The coefficient was used to measure the strength of the association between the urine BPA level and the parameters measuring semen quality, and its associated P value was used to determine whether the correlation was statistically significant. Because of the skewed distribution of urine BPA data, we included the variable in the regression model after $\log_{10}$-transformation. Before the $\log_{10}$-transformation, urine BPA values of 0 were input as LOD/($\sqrt{2}$), based on conventionally accepted practice (26).

To make the results more interpretable, we also measured the association using odds ratio (OR; i.e., the estimation of relative risk) based on dichotomized outcomes. Because the purpose of this epidemiologic study was to examine the general association between high urine BPA levels and the general trend of semen quality, and not necessarily to diagnose infertility, we did not use WHO cutoffs for abnormal semen quality, which are largely used for identifying clinical infertility. We dichotomized the parameters based on the median value of each parameter. Parameter values below the median were considered to be lower semen quality compared with parameter values above the median. We used logistic regression and its CI to examine the association between urine BPA levels and various parameters of lower semen quality after adjustment for potential confounders. We also examined the dose-response relationship using tertiles of urine BPA level, which does not require a linear assumption as in a linear regression model.

SUPPLEMENTARY FIGURE 1

Participation and inclusion: recruitment and criteria for study inclusion and exclusion. Ultimately, 218 men with both urine bisphenol-A (BPA) and semen quality measurements were included in the final analyses.



Li. Urine BPA level and semen quality. Fertil Steril 2011.

SUPPLEMENTARY TABLE 1

Distribution of urine bisphenol-A (BPA) by characteristics of the study population.

Characteristic	N[a] (218)	BPA (μg/gCr) Median	BPA (μg/gCr) Interquartile range (25^{th}–75^{th} percentiles)	*P* value
Age (y)				
≤25	14	21.0	(4.6–203.0)	.05
25–30	59	32.4	(6.1–104.9)	
30–35	60	10.6	(0.3–37.2)	
35–40	42	1.6	(0.0–37.0)	
40–45	21	19.8	(0.0–553.6)	
>45	22	26.6	(0.0–536.3)	
Education				
≤ Junior high	47	70.3	(0.0–2,769.0)	<.001
Senior high	125	16.7	(1.5–59.1)	
≥ College	46	1.7	(0.0–23.0)	
Married				
No	24	21.2	(4.5–79.1)	.47
Yes	194	14.2	(0.0–92.1)	
Employment history (y)				
<1	21	53.7	(8.2–203.0)	<.001
1–5	81	28.3	(1.5–168.0)	
≥5	116	6.6	(0.0–38.8)	
History of chronic disease[b]				
No	166	13.4	(0.2–95.9)	.63
Yes	52	19.1	(0.0–74.9)	
Age at first intercourse (y)				
<20	28	11.4	(3.1–47.5)	.21
≥20	184	14.2	(0.0–92.5)	
N/A[c]	6	120.4	(23.2–1,737.8)	
Ever exposed to other chemicals or heavy metals[d]				
No	108	10.5	(0.0–74.9)	.25
Yes	110	18.9	(1.1–95.9)	
Current smoker				
No	69	9.7	(0.1–172.6)	.65
Yes	149	17.6	(0.4–74.5)	
History of alcohol intake				
No	160	12.3	(0.2–82.8)	.47
Yes	58	29.1	(0.0-111.9)	
Occupational exposure to BPA				
No	88	1.4	(0.0–17.9)	<.001
Yes	130	38.7	(6.3–354.3)	
Steam bath/sauna use during past 2 wk				
No	210	15.2	(0.0–92.1)	.65
Yes	5	32.4	(3.6–37.9)	
History of subfertility[e]				
No	185	13.3	(0.0–75.2)	.98
Yes	16	14.4	(1.2–50.6)	

[a] The number in each category may not match the total number due to missing values.
[b] Diseases that may impact semen quality including urogenital diseases, autoimmune diseases, endocrine disorders, hypertension and other cardiovascular diseases, kidney diseases, and injury to genital organs.
[c] No history of sexual activity.
[d] Includes organic solvents, pesticides/herbicides, and heavy metals (e.g., lead, mercury, etc.).
[e] A history of unprotected intercourse on a regular basis for 12 months or longer without getting partner pregnant.

Li. Urine BPA level and semen quality. Fertil Steril 2011.

SUPPLEMENTARY TABLE 2

Distribution of semen parameters.

Semen parameter	N	Mean	Standard deviation	Median	Range
Concentration ($\times 10^6$ sperm/mL)	215	102.5	65.8	93.0	0.0–355.0
Total sperm count ($\times 10^6$)	215	297.9	242.9	252.0	0.0–1,530.0
Sperm vitality (% alive)	217	55.8	19.6	59.0	0.0–89.0
Sperm motility[a] (% moving forward)	218	39.2	15.0	42.0	0.0–72.0
Semen volume (mL)	218	2.9	1.3	2.7	0.2–7.2
Sperm morphology (% normal)	217	41.1	15.5	43.0	0.0–76.0

[a] A + B movement: Rapid (A) + slow (B) progressive (forward) movement.

Li. Urine BPA level and semen quality. Fertil Steril 2011.