

Differences in Bisphenol A and Estrogen Levels in the Plasma and Seminal Plasma of Men With Different Degrees of Infertility

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Summary

The general population is potentially exposed to many chemicals that can affect the endocrine system. These substances are called endocrine disruptors (EDs), and among them bisphenol A (BPA) is one of the most widely used and well studied. Nonetheless, there are still no data on simultaneous measurements of various EDs along with steroids directly in the seminal fluid, where deleterious effects of EDs on spermatogenesis and steroidogenesis are assumed. We determined levels of BPA and 3 estrogens using LC-MS/MS in the plasma and seminal plasma of 174 men with different degrees of infertility. These men were divided according their spermogram values into 4 groups: (1) healthy men, and (2) slightly, (3) moderate, and (4) severely infertile men. Estradiol levels differed across the groups and body fluids. Slightly infertile men have significantly higher BPA plasma and seminal plasma levels in comparison with healthy men ($p < 0.05$ and $p < 0.01$, respectively). Furthermore, seminal BPA, but not plasma BPA, was negatively associated with sperm concentration and total sperm count (-0.27 ; $p < 0.001$ and -0.24 ; $p < 0.01$, respectively). These findings point to the importance of seminal plasma in BPA research. Overall, a disruption of estrogen metabolism was observed together with a weak but significant impact of BPA on sperm count and concentration.

Key words

Bisphenol A • Estrone • Estradiol • Estriol • Seminal fluid/plasma
• Blood plasma • Infertile men • LC-MS

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Introduction

Bisphenol A (BPA) is a long- and well-known endocrine disruptor (ED), that still receives a considerable amount of attention from the scientific community as well as the general public, mainly because of its ubiquity in our environment and uncertainties about its effects on humans. For the most part, BPA enters the body by the ingestion of contaminated food or beverages. It leaks from polycarbonate plastics, which are used to line food and drink containers such as bottles and cans. Further minor ways of penetrating into the body are through the skin (e.g. contact with thermal receipts) (Ehrlich *et al.* 2014, Liao and Kannan 2011) or inhalation (e.g. cigarette smoke or dust) (Braun *et al.* 2011, He *et al.* 2009, Inoue *et al.* 2006, Rudel *et al.* 2003). There is still an ongoing debate whether environmental levels of BPA are harmful for the population or not.

BPA is a weak estrogen when considering its binding activities to the estrogen receptor (ER) (Welshons *et al.* 2003). On the other hand, it can act with the same potency as endogenous estradiol (E2) on the non-classical membrane estrogen receptor (Alonso-Magdalena *et al.* 2012, Quesada *et al.* 2002, Wozniak *et al.* 2005). Its mode of action, however, is much more complex. It may act through other nuclear receptors

including the estrogen related receptor (Delfosse *et al.* 2014, Okada *et al.* 2008), androgen receptor (Lee *et al.* 2003, Teng *et al.* 2013), thyroid receptor (Moriyama *et al.* 2002), glucocorticoid receptor (Sargis *et al.* 2010), peroxisome proliferator activated receptor γ (PPAR γ) (Pereira-Fernandes *et al.* 2013, Wang *et al.* 2010) and pregnane X receptor (Sui *et al.* 2012). An interaction of BPA with the expression and activity of steroidogenic enzymes has also been reported (Cannon *et al.* 2000, Gilibili *et al.* 2014, Hanioka *et al.* 1998, Ye *et al.* 2014, Ye *et al.* 2011, Zhang *et al.* 2011). Moreover, BPA exerts a non-monotonic dose response at low physiologically-relevant concentrations, with tissue-specific effects (for review see Wetherill *et al.* 2007).

Endogenous estrogens are thought to have an important role in the testis, because estrogen biosynthesis occurs in the testicular cells and the absence of ERs causes adverse effects on spermatogenesis as well as steroidogenesis (Akingbemi 2005). Physiological levels of E2 are essential for normal spermatogenesis; in contrast, a surplus of estrogens (together with a lack of testosterone) occurs in infertility (Pavlovich *et al.* 2001). The impact of BPA on male reproductive function is of particular interest due to its estrogenic and antiandrogenic activities, which could have potentially deleterious effects on spermatogenesis (reviewed in Hampl *et al.* 2013a,b).

Recently, our group developed a sensitive and accurate method for the simultaneous measurement of estrogens (estrone, estradiol and estriol) and BPA in human plasma and seminal fluid (Vitku *et al.* 2015). Reported levels of estrogens and BPA vary in these biological fluids in normospermic men, underlining the fact that seminal fluid is a unique environment where the effects of BPA may be expressed directly in the testis. In this study, we aimed to investigate BPA and estrogen concentrations in the plasma and seminal fluid in men with different degrees of infertility, and evaluate the potential effects of BPA on the estrogen metabolism and sperm quality.

Materials and Methods

Chemicals and reagents

Reference standards of estrone (E1), 17 β -estradiol (E2) and estriol (E3) and deuterated standards of estrone (d4E1) and estriol (d2E3) were purchased from Steraloids (Newport, RI, USA). Bisphenol A (BPA), deuterated BPA (d16BPA) and deuterated E2 (d3E2) were obtained from Sigma-Aldrich (St. Louis, MO, USA)

as were 99.9 % tert-butyl methyl ether (MTBE), acetone, sodium bicarbonate, sodium hydroxide and dansyl chloride. Methanol and water for chromatography were purchased from Merck (Darmstadt, Germany). All solvents and reagents were of HPLC grade.

Study population and sample collection

Samples of plasma and seminal plasma were obtained from patients attending the Centre of Assisted Reproduction Pronatal (Prague, CZ). Each patient underwent a standardized ejaculate examination (spermiogram) according to the World Health Organization (WHO) 2010 criteria. In a previous study (Vitku *et al.* 2015), we dealt with the problem of ensuring that sampling equipment is not contaminated with BPA. Following the procedures detailed in that study, all steps in sample collection and processing were carried out using BPA-free glass equipment and stored in glass tubes in -20°C until analysis. Plasma and seminal plasma samples were obtained from 174 men with different degrees of infertility. The mean age of the men was 35.97 ± 5.64 years and BMI 27.32 ± 3.65 . Men were divided into four groups according to spermiogram values. The first group included normospermic men with a normal spermiogram (n=84); oligospermic, asthenospermic and oligoasthenospermic men were included in the second group (n=56); teratospermic, oligoasthenoteratospermic and oligoteratospermic men were placed in the third group (n=20); and the fourth group consisted of azospermic men (n=14). We termed these groups: (1) healthy men, and (2) slightly, (3) moderately and (4) severely infertile men.

The study was performed in accordance with the Declaration of Helsinki (2000) of the World Medical Association. The protocol was approved by the Ethical Committee of the Institute of Endocrinology. Informed and written consent with the use of biological materials for research reasons was obtained from all subjects participating in the project.

Determination of estrogens and BPA

We analyzed unconjugated forms of E1, E2, E3 and BPA in plasma and seminal plasma by a newly developed isotope dilution ultra high performance liquid chromatography – mass spectrometry method (Vitku *et al.* 2015). A Kinetex C18 column (100 x 3.0 mm, 1.7 μm ; Phenomenex, Torrance, CA, USA) and Security Guard ULTRA cartridge system (UHPLC C18 for 3 mm ID column; Phenomenex, Torrance, CA, USA) was used for

the analysis. An Eksigent ultraLC 110 liquid chromatograph system (Redwood City, CA, USA) was coupled to an API 3200 mass spectrometer (AB Sciex, Concord, Canada) with an electrospray ionization (ESI) probe operating in positive mode.

Detailed information about the analysis procedure and validation are provided elsewhere (Vitku *et al.* 2015). Briefly, 500 µl of plasma or 1000 µl of seminal fluid was diluted by 500 µl of physiological solution (0.9 % sodium chloride) and samples were vortexed. Consequently, extraction with 2 ml of 99.9 % tert-butyl methyl ether (MTBE) for one minute was performed. The organic phase was evaporated until dryness using a vacuum concentrator (55 °C). Further, a derivatization step was carried out: a volume of 50 µl of sodium bicarbonate buffer (100 mM, pH 10.5) and 50 µl of dansyl chloride in acetone (1 mg/ml) were added to the dry residues, shortly vortexed and incubated in a heat block (60 °C) for 5 min. After removing from the heat block, samples were left to cool down to room temperature and again evaporated until dryness. Thereafter, samples were reconstituted with 300 µl of methanol, and 50 µl were transferred to the glass insert containing 50 µl of the ammonium formate in ultrapure water (10 mM) pre-pipetted. The injection volume was 50 µl.

Statistical evaluation

Before statistical analysis, the data were transformed by Box-Cox transformation due to the significant skewness, kurtosis and heteroscedasticity of most variables. Differences between groups were evaluated using one-way ANOVA followed by least square difference multiple comparisons. The statistical software Statgraphics Centurion XVI from Statpoint Inc. (Warrenton, VA, USA) was used for data transformations, correlations, ANOVA testing and multiple comparisons. Multiple outliers for correlations were found using NCSS 2007 (Kaysville, UT, USA).

Results

Here we report the first data on BPA exposure in a population of Czech men (Table 1). The groups of men did not significantly differ from each other in age and BMI. We detected BPA, E2, E1 and E3 in 87 %, 94 %, 100 % and 62 % of plasma samples, respectively, and 92 %, 84 %, 90 % and 97 % of seminal samples, respectively. In the rest of the samples, levels were under the lower limit of quantification.

Table 1. Comparisons of age, BMI, BPA (pg/ml) and estrogen levels (pg/ml) in groups of men with different degrees of infertility.

		Group 1 (n=84)	Group 2 (n=56)	Group 3 (n=20)	Group 4 (n=14)	p-value	Multiple comparisons
	Age	36 (34.9; 36.7)	35.8 (34.5; 37.2)	35.7 (33.4; 37.9)	35.2 (32.5; 37.9)	0.959	
	BMI	27.4 (26.2;28.5)	27.5 (26.2;28.7)	26.6 (24.9; 28.2)	26.2 (24.9; 28.2)	0.451	
<i>Plasma</i>	BPA	47 (26;81)	137 (75; 239)	114 (42; 270)	33 (6;125)	0.036	1<2
	E2	22 (18;26)	18 (15;23)	17 (11;24)	7 (3;12)	0.002	1,2,3>4
	E1	24 (20;29)	23 (18;28)	21 (15;29)	17 (10;26)	0.513	
	E3	19 (11;31)	20 (10;36)	19 (5;53)	13 (3;36)	0.933	
<i>Seminal fluid</i>	BPA	66 (44;94)	144 (98;205)	132 (69;228)	179 (84;330)	0.009	1<2,4
	E2	2 (1;3)	4 (3;6)	9 (5;15)	7 (3;14)	0.002	1<3,4; 2<3
	E1	4 (3;5)	5 (4;7)	9 (6;13)	6 (3;9)	0.008	1,2<3
	E3	43 (30;59)	31 (19;47)	34 (16;63)	83 (40;154)	0.129	2<4

Data are shown as means and 95.0 percent confidence intervals (in parentheses) for each group, with levels of significance for the ANOVA and multiple comparisons provided. Group 1 = normospermic men; Group 2 = oligospermic/asthenospermic/oligoasthenospermic men; Group 3 = teratospermic/oligoteratospermic/oligoasthenoteratospermic men; Group 4 = azospermic men.

Comparisons of plasma and seminal BPA and estrogen levels in men with different degrees of infertility

Generally, BPA levels in seminal fluid were slightly higher than those in plasma, except for in the 4th

group where seminal fluid BPA levels were nearly three times greater (Table 1). Seminal BPA concentrations were found to be higher in all groups of men with various degrees of infertility in comparison with normospermic

men. Plasma BPA levels were significantly higher in the group of slightly infertile men compared to healthy men. Concentrations of plasma E2 decreased from the first to the fourth group, while seminal E2 levels increased. Mean plasma levels of E2 varied from 7 to 22 pg/ml among the groups, in comparison with 2-7 pg/ml mean E2 in semen. Plasma and seminal fluid E1 levels showed similar results as for E2. Concentrations of E3 in seminal fluid were significantly higher than E3 plasma levels in all groups, and did not differ across the groups. A graphical representation of the differences in BPA and E2 levels in both fluids across the groups is shown in

Figure 1.

Correlation matrix between BPA, estrogens and spermogram parameters

Pearson's correlation coefficients between all parameters are provided in Table 2. Although plasma and seminal BPA levels correlate with each other, only BPA in seminal fluid was negatively associated with sperm concentration and total sperm count. This indicates the importance of seminal fluid in research on the effects of BPA. Another finding that is of interest is the positive association between BPA and E2 in both body fluids.

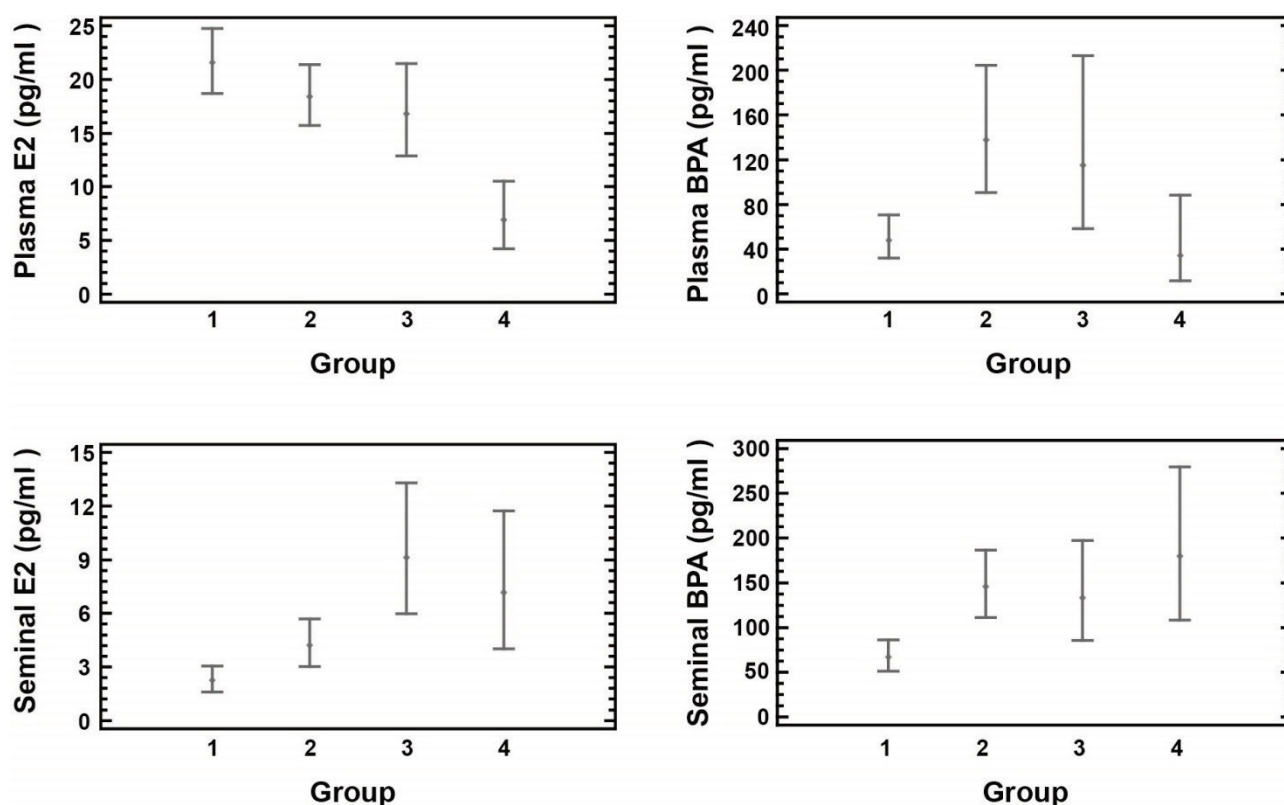


Fig. 1. Differences in bisphenol A (BPA) and estradiol (E2) levels in plasma and seminal plasma among the 4 groups of men. Group 1 = normospermic men (n=84); Group 2 = oligospermic/asthenospermic/oligoasthenospermic men (n=56); Group 3 = teratospermic/oligoteratospermic/oligoasthenoteratospermic men (n=20); Group 4= azospermic men (n=14).

Discussion

To the best of our knowledge, this is the first study reporting levels of EDs and steroids directly in seminal fluid. We measured unconjugated forms of estrogens as well as BPA, thought to be the active forms, and found that the levels differ in seminal fluid and plasma. This indicates that seminal fluid is a unique milieu that deserves further investigation.

Experimental studies in adult rodents have

provided evidence that exposure to BPA affects sperm quality and production (reviewed in Peretz *et al.* 2014). However, few studies have reported the impact of BPA on sperm quality in adult men. To our knowledge, six studies have dealt with this problem, all measuring BPA in urine, and with divergent results (Goldstone *et al.* 2014, Knez *et al.* 2014, Lassen *et al.* 2014, Li *et al.* 2011, Meeker *et al.* 2010b, Mendiola *et al.* 2010). Our finding of decreasing sperm concentrations and counts in association with increasing seminal BPA (Table 2) is in

agreement with three of the studies (Knez *et al.* 2014, Li *et al.* 2011, Meeker *et al.* 2010b). In contrast, the other studies did not find any association between these sperm parameters and urinary BPA (Goldstone *et al.* 2014, Mendiola *et al.* 2010). However, Lassen *et al.* (2014)

reported a significant inverse association between BPA excretion and progressive motility. Still, the outcomes vary across studies, apparently due to the difficulties in evaluating the effects of EDs when organisms are exposed more than one at a time.

Table 2. Pearson's correlation coefficients among levels of plasma and seminal BPA and estrogens, and spermogram parameters. Correlation coefficients are provided in the first row of each variable, p-values are in the second row. If significant, coefficients and p-values are highlighted in bold.

	Plasma E2	Plasma E1	Plasma E3	Plasma BPA	Seminal E2	Seminal E1	Seminal E3	Seminal BPA	Concentration	Total count	Motility	Progressive motility	Morphology
<i>Plasma E2</i>	1.000												
<i>Plasma E1</i>	0.372 0.000	1.000											
<i>Plasma E3</i>	0.366 0.001	0.173 0.144	1.000										
<i>Plasma BPA</i>	0.363 0.000	-0.146 0.120	0.192 0.103	1.000									
<i>Seminal E2</i>	-0.065 0.498	-0.236 0.012	0.190 0.110	0.076 0.378	1.000								
<i>Seminal E1</i>	0.035 0.714	-0.004 0.967	0.124 0.299	0.058 0.499	0.181 0.023	1.000							
<i>Seminal E3</i>	0.229 0.016	-0.059 0.538	0.384 0.001	0.106 0.218	0.192 0.016	0.158 0.047	1.000						
<i>Seminal BPA</i>	0.082 0.395	-0.257 0.006	0.185 0.120	0.338 0.000	0.318 0.000	0.061 0.450	0.156 0.051	1.000					
<i>Concentration</i>	-0.006 0.952	0.053 0.575	-0.003 0.983	-0.160 0.057	-0.146 0.066	-0.098 0.222	0.063 0.429	-0.271 0.001	1.000				
<i>Total count</i>	-0.059 0.536	0.077 0.412	-0.022 0.852	-0.163 0.052	-0.164 0.039	-0.106 0.184	0.010 0.898	-0.236 0.003	0.957 0.000	1.000			
<i>Motility</i>	0.293 0.002	0.164 0.081	0.268 0.022	0.065 0.439	-0.248 0.002	-0.187 0.019	0.021 0.796	-0.115 0.151	0.513 0.000	0.506 0.000	1.000		
<i>Progressive motility</i>	0.291 0.002	0.172 0.066	0.231 0.049	0.012 0.886	-0.244 0.002	-0.225 0.005	0.027 0.732	-0.129 0.106	0.546 0.000	0.550 0.000	0.958 0.000	1.000	
<i>Morphology</i>	0.096 0.310	-0.010 0.918	0.133 0.264	-0.103 0.223	-0.203 0.010	-0.185 0.020	0.013 0.870	-0.124 0.119	0.595 0.000	0.561 0.000	0.722 0.000	0.741 0.000	1.000

The levels of BPA in the plasma of group 4 are similar to those of group 1, i.e. of normal fertile men. One possible reason may be that the severe infertility in men of group 4 is caused by other factors than the effects of hormones and other constituents of seminal fluid, e.g. genetic or anatomic causes or the result of infections.

Although the body of ED research is continuously expanding, there still exist uncertainties in the process of BPA degradation in the body. It has generally been thought that BPA is rapidly metabolized in the liver and excreted in the urine within hours (Volkel *et al.* 2002, 2005). On the other hand, a recent study

showed that during fasting BPA levels did not decline rapidly, suggesting a substantial non-food-related exposure or accumulation in tissues (Stahlhut *et al.* 2009). This is why we decided to study the relationships between EDs and sperm quality in seminal fluid, with its closer proximity to sperm production. According to our previous study, seminal and plasma BPA levels in normospermic men did not correlate with each other, indicating that their distribution or metabolism are different in these body fluids (Vitku *et al.* 2015). Furthermore, BPA competes with sex steroids for human plasma SHBG (Dechaud *et al.* 1999), suggesting that the bioavailability and half-time in blood could be affected. Further studies on the persistence of BPA in semen and other body fluids during chronic exposure are needed.

Studies that have investigated associations between BPA and steroidogenesis are divergent as well. Only a few of them have investigated the impact of BPA on estrogen levels and estrogen metabolism. Urinary BPA was reported to be positively associated with plasma E2 in a group of young men (Lassen *et al.* 2014). Another study showed no association with E2 levels (Galloway *et al.* 2010) and another reported an inverse relationship (Meeker *et al.* 2010a).

Our results show that the levels of E2 differ across the groups and body fluids. Only 10-25 % of the E2 in circulation in men is synthesized in the testis. Aromatase activity and estrogen biosynthesis in men occur mainly in adipose tissue (Levine *et al.* 1997). In our study, the increases of E2 and E1 in the seminal plasma of increasingly infertile men and the opposite trends in plasma raise some considerations. One explanation is that peripheral estrogens penetrate more easily through the

blood-testis-barrier to the testis. Alternatively, estrogens originating in the testis may have a harder time accessing the periphery. Other possibilities include the increased expression or activity of aromatase in the testis and/or decreased expression or activity in the adipose tissue. The metabolism of E2 could be also protracted by the interaction of BPA with enzymes involved in steroid conjugation such as estrogen glucuronidase or sulfotransferase. This explanation is in accordance with the *in vitro* study of Zhang *et al.* (2011), who reported that BPA suppressed E2 catabolism in H295R cells but without altering aromatase activity. On the other hand, studies from *in vitro* and *in vivo* experiments focused on the impact of BPA on aromatase activity have yielded conflicting results (Castro *et al.* 2013, Ehrlich *et al.* 2013, Rajakumar *et al.* 2015, Shanthanagouda *et al.* 2014).

In conclusion, the results of our study show an inverse association between seminal BPA and sperm count and concentration. The correlation coefficients were relatively weak ($r=-0.24$ and $r=-0.27$, respectively) suggesting that BPA slightly, but significantly, contributes to the final state of sperm quality, together with other factors. Moreover, a disruption of estrogen metabolism was observed, but the mechanism of action remains to be elucidated.

Conflict of Interest

There is no conflict of interest.

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References

- AKINGBEMI BT: Estrogen regulation of testicular function. *Reprod Biol Endocrinol* **3**: 51, 2005.
- ALONSO-MAGDALENA P, ROPERO AB, SORIANO S, GARCIA-AREVALO M, RIPOLL C, FUENTES E, QUESADA I, NADAL A: Bisphenol-A acts as a potent estrogen via non-classical estrogen triggered pathways. *Mol Cell Endocrinol* **355**: 201-207, 2012.
- BRAUN JM, KALKBRENNER AE, CALAFAT AM, BERNERT JT, YE X, SILVA MJ, BARR DB, SATHYANARAYANA S, LANPHEAR BP: Variability and predictors of urinary bisphenol A concentrations during pregnancy. *Environ Health Perspect* **119**: 131-137, 2011.
- CANNON JM, KOSTORYZ E, RUSSO KA, SMITH RE, YOURTEE DM: Bisphenol A and its biomaterial monomer derivatives alteration of *in vitro* cytochrome P450 metabolism in rat, minipig, and human. *Biomacromolecules* **1**: 656-664, 2000.
- CASTRO B, SANCHEZ P, TORRES JM, PREDA O, DEL MORAL RG, ORTEGA E: Bisphenol A exposure during adulthood alters expression of aromatase and 5 α -reductase isozymes in rat prostate. *PLoS One* **8**: e55905, 2013.

- DECHAUD H, RAVARD C, CLAUSTRAT F, DE LA PERRIERE AB, PUGEAT M: Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). *Steroids* **64**: 328-334, 1999.
- DELFOSSÉ V, GRIMALDI M, LE MAIRE A, BOURGUET W, BALAGUER P: Nuclear receptor profiling of bisphenol-a and its halogenated analogues. *Vitam Horm* **94**: 229-251, 2014.
- EHRlich S, WILLIAMS PL, HAUSER R, MISSMER SA, PERETZ J, CALAFAT AM, FLAWS JA: Urinary bisphenol A concentrations and cytochrome P450 19 A1 (Cyp19) gene expression in ovarian granulosa cells: an in vivo human study. *Reprod Toxicol* **42**: 18-23, 2013.
- EHRlich S, CALAFAT AM, HUMBLET O, SMITH T, HAUSER R: Handling of thermal receipts as a source of exposure to bisphenol A. *JAMA* **311**: 859-860, 2014.
- GALLOWAY T, CIPELLI R, GURALNIK J, FERRUCCI L, BANDINELLI S, CORSI AM, MONEY C, MCCORMACK P, MELZER D: Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. *Environ Health Perspect* **118**: 1603-1608, 2010.
- GILIBILI RR, VOGL AW, CHANG TK, BANDIERA SM: Localization of cytochrome P450 and related enzymes in adult rat testis and downregulation by estradiol and bisphenol A. *Toxicol Sci* **140**: 26-39, 2014.
- GOLDSTONE AE, CHEN Z, PERRY MJ, KANNAN K, LOUIS GM: Urinary bisphenol A and semen quality, the LIFE Study. *Reprod Toxicol* **51C**: 7-13, 2014.
- HAMPL R, KUBATOVA J, HERACEK J, SOBOTKA V, STARKA L: Hormones and endocrine disruptors in human seminal plasma. *Endocr Regul* **47**: 149-158, 2013a.
- HAMPL R, KUBATOVA J, SOBOTKA V, HERACEK J: Steroids in semen, their role in spermatogenesis, and the possible impact of endocrine disruptors. *Horm Mol Biol Clin Investig* **13**: 1-5, 2013b.
- HANIOKA N, JINNO H, NISHIMURA T, ANDO M: Suppression of male-specific cytochrome P450 isoforms by bisphenol A in rat liver. *Arch Toxicol* **72**: 387-394, 1998.
- HE Y, MIAO M, HERRINTON LJ, WU C, YUAN W, ZHOU Z, LI DK: Bisphenol A levels in blood and urine in a Chinese population and the personal factors affecting the levels. *Environ Res* **109**: 629-633, 2009.
- INOUE K, YOSHIDA S, NAKAYAMA S, ITO R, OKANOUCI N, NAKAZAWA H: Development of stable isotope dilution quantification liquid chromatography-mass spectrometry method for estimation of exposure levels of bisphenol A, 4-tert-octylphenol, 4-nonylphenol, tetrabromobisphenol A, and pentachlorophenol in indoor air. *Arch Environ Contam Toxicol* **51**: 503-508, 2006.
- KNEZ J, KRANVOGL R, BREZNIK BP, VONCINA E, VLAISAVLJEVIC V: Are urinary bisphenol A levels in men related to semen quality and embryo development after medically assisted reproduction? *Fertil Steril* **101**: 215-221, e1-e5, 2014.
- LASSEN TH, FREDERIKSEN H, JENSEN TK, PETERSEN JH, JOENSEN UN, MAIN KM, SKAKKEBAEK NE, JUUL A, JORGENSEN N, ANDERSSON AM: Urinary bisphenol A levels in young men: association with reproductive hormones and semen quality. *Environ Health Perspect* **122**: 478-484, 2014.
- LEE HJ, CHATTOPADHYAY S, GONG EY, AHN RS, LEE K: Antiandrogenic effects of bisphenol A and nonylphenol on the function of androgen receptor. *Toxicol Sci* **75**: 40-46, 2003.
- LEVINE AC, KIRSCHENBAUM A, GABRILOVE JL: The role of sex steroids in the pathogenesis and maintenance of benign prostatic hyperplasia. *Mt Sinai J Med* **64**: 20-25, 1997.
- LI DK, ZHOU Z, MIAO M, HE Y, WANG J, FERBER J, HERRINTON LJ, GAO E, YUAN W: Urine bisphenol-A (BPA) level in relation to semen quality. *Fertil Steril* **95**: 625-630, e1-e4, 2011.
- LIAO C, KANNAN K: Widespread occurrence of bisphenol A in paper and paper products: implications for human exposure. *Environ Sci Technol* **45**: 9372-9379, 2011.
- MEEKER JD, CALAFAT AM, HAUSER R: Urinary bisphenol A concentrations in relation to serum thyroid and reproductive hormone levels in men from an infertility clinic. *Environ Sci Technol* **44**: 1458-1463, 2010a.
- MEEKER JD, EHRlich S, TOTH TL, WRIGHT DL, CALAFAT AM, TRISINI AT, YE X, HAUSER R: Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. *Reprod Toxicol* **30**: 532-539, 2010b.

- MENDIOLA J, JORGENSEN N, ANDERSSON AM, CALAFAT AM, YE X, REDMON JB, DROBNIS EZ, WANG C, SPARKS A, THURSTON SW, LIU F, SWAN SH: Are environmental levels of bisphenol a associated with reproductive function in fertile men? *Environ Health Perspect* **118**: 1286-1291, 2010.
- MORIYAMA K, TAGAMI T, AKAMIZU T, USUI T, SAIJO M, KANAMOTO N, HATAYA Y, SHIMATSU A, KUZUYA H, NAKAO K: Thyroid hormone action is disrupted by bisphenol A as an antagonist. *J Clin Endocrinol Metab* **87**: 5185-5190, 2002.
- OKADA H, TOKUNAGA T, LIU X, TAKAYANAGI S, MATSUSHIMA A, SHIMOHIGASHI Y: Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-gamma. *Environ Health Perspect* **116**: 32-38, 2008.
- PAVLOVICH CP, KING P, GOLDSTEIN M, SCHLEGEL PN: Evidence of a treatable endocrinopathy in infertile men. *J Urol* **165**: 837-841, 2001.
- PEREIRA-FERNANDES A, DEMAEGDT H, VANDERMEIREN K, HECTORS TL, JORENS PG, BLUST R, VANPARYS C: Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. *PLoS One* **8**: e77481, 2013.
- PERETZ J, VROOMAN L, RICKE WA, HUNT PA, EHRLICH S, HAUSER R, PADMANABHAN V, TAYLOR HS, SWAN SH, VANDEVOORT CA, FLAWS JA: Bisphenol a and reproductive health: update of experimental and human evidence, 2007-2013. *Environ Health Perspect* **122**: 775-786, 2014.
- QUESADA I, FUENTES E, VISO-LEON MC, SORIA B, RIPOLL C, NADAL A: Low doses of the endocrine disruptor bisphenol-A and the native hormone 17beta-estradiol rapidly activate transcription factor CREB. *FASEB J* **16**: 1671-1673, 2002.
- RAJAKUMAR C, GUAN H, LANGLOIS D, CERNEA M, YANG K: Bisphenol A disrupts gene expression in human placental trophoblast cells. *Reprod Toxicol* **53**: 39-44, 2015.
- RUDEL RA, CAMANN DE, SPENGLER JD, KORN LR, BRODY JG: Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environ Sci Technol* **37**: 4543-4553, 2003.
- SARGIS RM, JOHNSON DN, CHOUDHURY RA, BRADY MJ: Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. *Obesity (Silver Spring)* **18**: 1283-1288, 2010.
- SHANTHANAGOUDA AH, NUGEGODA D, PATIL JG: Effects of bisphenol A and fadrozole exposures on cyp19a1 expression in the Murray rainbowfish, *Melanotaenia fluviatilis*. *Arch Environ Contam Toxicol* **67**: 270-280, 2014.
- STAHLHUT RW, WELSHONS WV, SWAN SH: Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. *Environ Health Perspect* **117**: 784-789, 2009.
- SUI Y, AI N, PARK SH, RIOS-PILIER J, PERKINS JT, WELSH WJ, ZHOU C: Bisphenol A and its analogues activate human pregnane X receptor. *Environ Health Perspect* **120**: 399-405, 2012.
- TENG C, GOODWIN B, SHOCKLEY K, XIA M, HUANG R, NORRIS J, MERRICK BA, JETTEN AM, AUSTIN CP, TICE RR: Bisphenol A affects androgen receptor function via multiple mechanisms. *Chem Biol Interact* **203**: 556-564, 2013.
- VITKU J, CHLUPACOVA T, SOSVOROVA L, HAMPL R, HILL M, HERACEK J, BICIKOVA M, STARKA L: Development and validation of LC-MS/MS method for quantification of bisphenol A and estrogens in human plasma and seminal fluid. *Talanta* **140**: 62-67, 2015.
- VOLKEL W, COLNOT T, CSANADY GA, FILSER JG, DEKANT W: Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. *Chem Res Toxicol* **15**: 1281-1287, 2002.
- VOLKEL W, BITTNER N, DEKANT W: Quantitation of bisphenol A and bisphenol A glucuronide in biological samples by high performance liquid chromatography-tandem mass spectrometry. *Drug Metab Dispos* **33**: 1748-1757, 2005.
- WANG YF, CHAO HR, WU CH, TSENG CH, KUO YT, TSOU TC: A recombinant peroxisome proliferator response element-driven luciferase assay for evaluation of potential environmental obesogens. *Biotechnol Lett* **32**: 1789-1796, 2010.

- WELSHONS WV, THAYER KA, JUDY BM, TAYLOR JA, CURRAN EM, VOM SAAL FS: Large effects from small exposures. I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environ Health Perspect* **111**: 994-1006, 2003.
- WETHERILL YB, AKINGBEMI BT, KANNO J, McLACHLAN JA, NADAL A, SONNENSCHNEIN C, WATSON CS, ZOELLER RT, BELCHER SM: In vitro molecular mechanisms of bisphenol A action. *Reprod Toxicol* **24**: 178-198, 2007.
- WOZNIAK AL, BULAYEVA NN, WATSON CS: Xenoestrogens at picomolar to nanomolar concentrations trigger membrane estrogen receptor-alpha-mediated Ca²⁺ fluxes and prolactin release in GH3/B6 pituitary tumor cells. *Environ Health Perspect* **113**: 431-439, 2005.
- YE L, ZHAO B, HU G, CHU Y, GE RS: Inhibition of human and rat testicular steroidogenic enzyme activities by bisphenol A. *Toxicol Lett* **207**: 137-142, 2011.
- YE L, GUO J, GE RS: Environmental pollutants and hydroxysteroid dehydrogenases. *Vitam Horm* **94**: 349-390, 2014.
- ZHANG X, CHANG H, WISEMAN S, HE Y, HIGLEY E, JONES P, WONG CK, AL-KHEDHAIRY A, GIESY JP, HECKER M: Bisphenol A disrupts steroidogenesis in human H295R cells. *Toxicol Sci* **121**: 320-327, 2011.
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