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Effect of Bisphenol A on Luteinizing Hormones and Follicle-Stimulating Hormone in Female Rats

[1]E. Oguazu Chinenye, [1]C. Ezeonu Francis, [2]Dike C. Charles, [1]Ubaoji K. Ikechukwu and [1]M.O. Enemali
[1]Department of Applied Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria
[2]Department of Human Biochemistry, College of Basic Health Sciences, Nnamdi Azikiwe University, Nnewi, Nigeria

ABSTRACT

Background and Objective: BPA is disruptive to women's hormone homeostasis. Environmentally relevant doses of BPA can cause effects on human development and reproduction. BPA can impair implantation and impair the establishment of pregnancies leading to a high rate of infertility. BPA may stimulate cell proliferation and stimulates uterine, vaginal and mammary growth. This study investigates the possible effect of BPA on LH and FSH at prevailing low exposure rates in albino Wistar rats. **Materials and Methods:** Ten experimental groups each containing 10 female rats were administered 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg of BPA/kg body weight, respectively for 3 months. The eleventh control group was given water. Animal blood was collected at the end of every week of the studies and the serum sample specimens were analyzed by routine diagnostic procedures for the parameters assayed. **Results:** Significantly increased concentrations of luteinizing hormones were observed at all concentrations of BPA exposure and FSH was decreased. **Conclusion:** The present study is evident that BPA exposure alters the reproductive hormones and this implies that BPA can alter steroid hormone pathways.

KEYWORDS

BPA, luteinizing hormones, Follicle-Stimulating Hormone (FSH), homeostasis, female, exposure, reproductive system

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INTRODUCTION

Bisphenol-A (BPA) is one of the world's highest production volume chemicals[1] used in the production of polycarbonate plastics and epoxy resins found in baby bottles, water supply, the lacquer lining of the can, dental sealants, carbonless copy paper and thermal paper[2] and can leach from these polymers into water or food products[3].

Bisphenol A (BPA) has increasingly been receiving 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, penetrating the body through the skin[4,5] and inhalation of contaminated dust[6,7]. These disturbing facts raise questions about the extent to which current, widespread exposures to BPA are contributing to the burden of infertility and reproductive health challenges.

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BPA is capable of inducing toxic effects on vital organs, several studies have reported that absorption of BPA has caused extensive damage to the liver and kidney[8,9], formation of multinucleated giant cells in the liver hepatocytes, DNA adduct formation and induced the production of free radicals in hepatocytes[10,11]. Indeed, this chemical compound may be involved in adipose tissue dysfunction, metabolic/endocrine dysfunctions, cancer and fertility problems[12-14], impaired plasma glucose[15], involved in insulin resistance[16], causes permanent chromosomal damage linked to recurrent miscarriage and birth defects[17], spur both the formation and growth of fat cells[18]. It has been reported that BPA can be detected in more than 90% of people in population-representative samples[19]. Bisphenol-A is considered to be an endocrine-disrupting chemical with reproductive toxicity and oxidation of lipids[20,21]. Rodent and in vitro studies have suggested that BPA has both estrogenic and anti-androgenic effects[22,23]. Bousoumah *et al*.[24], reported that urinary concentrations of BPA were significantly higher in exposed workers than in controls.

This study aims to evaluate the possible effects of bisphenol A on sex hormone levels in female Wistar albino rats, serum Follicle-Stimulating Hormone (FSH) and LH measured.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Biochemistry, Research Laboratory, Faculty of Natural and Applied Sciences, Gregory University, Uturu, Abia State, Nigeria from June to August, 2021.

Experimentation: One hundred and ten non-pregnant female rats of age 5 weeks were acclimatized in the laboratory for seven days and randomly divided into 11 groups experimental of 10 rats each and respectively administered 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1 mg of BPA/kg b.wt./day. The first group which served as control did not receive any treatment but distilled water instead. The graded doses of BPA were dissolved in distilled water and administered by oral gavage using an intubation canular. Blood was obtained from the tail of the various groups by capillary action weekly, after BPA administration for 13 weeks. Blood samples were processed for clinical assay.

Animals have housed in aluminium wire-mesh cages in a well-ventilated animal house with a 12 hrs dark/light cycle and at room temperature and were provided commercial rat pellets (Vital feed from Vital Group of Company, Nigeria) and water *ad libitum*.

At the end of the experiments, serum FSH and LH were assayed using Chemwell 2910 Auotanalyser. All reagents were commercially obtained as already prepared kits. The kits for FSH and LH were purchased from the Egyptian Company for Biotechnology (SAE) in Cairo, Egypt. Individual tests were carried out according to the kit specifications as described below.

Luteinizing Hormone (LH) assay

Principle: The Principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich-type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for LH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of LH is conjugated to Horse Radish Peroxidase (HRP). LH from the sample and blank is allowed to bind to the plate, washed and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by the addition of the stopping solution.

The concentration is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of LH in the sample.

Procedure: The required number of microwells strips was placed in the holder and 25 µL of each calibrator, control and specimen sample were dispensed into correspondingly labelled wells. Then,

100 µL of assay buffer was pipetted into each well and incubated for 30 min at 25°C. The wells were washed 3 times with 300 µL of diluted wash buffer per well and tapped firmly against absorbent paper to ensure that it is dry. Another 100 µL of the conjugate working solution was pipetted into each well and incubated for 30 min at 25°C. The plate wells were washed again in the same manner as stated earlier. Then, 100 µL of TMB substrate was dispensed into each well and incubated for 20 min at 25°C. Finally, 50 µL of stop solution was pipetted into each well. The concentration was measured at 450 nm within 20 min after the addition of the stop solution.

Follicle-Stimulating Hormone (FSH) assay

Principle: The Principle of the following enzyme immunoassay test follows a typical two-step capture or "sandwich" type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for FSH is immobilized onto the microwell plate and another monoclonal antibody specific for a different region of FSH is conjugated to Horse Radish Peroxidase (HRP). FSH from the sample and blank is allowed to bind to the plate, washed and subsequently incubated with the HRP conjugate. After a second washing step, the enzyme substrate is added. The enzymatic reaction is terminated by the addition of the stop solution. The concentration is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of FSH in the sample.

Procedure: The required number of microwell strips was placed in the holder and 25 µL of each calibrator, control and specimen sample were pipetted into correspondingly labelled wells. Then, 100 µL of assay buffer was added to each well and incubated for 30 min at 25°C. The wells were washed 3 times with 300 µL of diluted wash buffer per well and tapped firmly against absorbent paper to ensure that it is dry. Again, 100 µL of the conjugate working solution was added to each well and incubated for 30 min at 25°C. The washing of the wells was repeated in the same manner. Another 100 µL of TMB substrate was pipetted into each well and incubated on a plate shaker for 20 min at 25°C. Then, 50 µL of stop solution was added to each well to stop the reaction. The concentrations were read at 450 nm within 20 min after the addition of the stop solution.

Statistical analysis: Differences between obtained values (Mean±SD) were carried out by One-way Analysis of Variance (ANOVA) using SPSS software version 20.0 followed by the Tukey-Kramer Multiple Comparison Test. A $p \leq 0.05$ was taken as a criterion for a statistically significant difference.

RESULTS

Effect on luteinizing hormone: There is a significant dose-dependent increase in the luteinizing hormone level when compared with the control at $p \leq 0.05$. Group 0.1 mg kg^{-1} at the first 4 weeks of the experiment declined below the control level (Fig. 1a). This was followed by a steady increase with time (Fig. 1b). The peak effect was observed at group 0.6 mg kg^{-1} and then a slight decline which is sharp at group 0.9 mg kg^{-1}, the maximum effect was observed at group 1 mg kg^{-1} (see Fig. 1c). Again, an increase at lower doses does not appear to be dose-dependent. Doses above 0.05 mg kg^{-1} showed a dose-dependent increase in LH up to 0.6 mg kg^{-1}, after which there was a decline up to 0.9 mg kg^{-1} and then spiked at the dose of 1 mg kg^{-1}. In all instances, the LH level increased with time, week 13 showed the maximum effect except at dose group 0.3 mg kg^{-1} where is a time-dependent decrease from week 9-13 (Fig. 1c). The BPA groups with the high level of LH are group 0.6 and 1 mg kg^{-1} (Fig 1a-c). There is a steady increase up to 0.6 mg kg^{-1} group, then the LH levels began to decrease at group 0.7 mg kg^{-1} through to group 0.9 mg kg^{-1} and then it starts to rise again. Although none of these decreases falls below the control level except for group 0.1 mg kg^{-1} at week 2 through to week 3 (Fig 1a). Dose 0.1 mg kg^{-1} slightly decline below the control at week-1-2 (Fig. 1a), while in we week 3 and 4 (Fig. 1a) it showed no significance in the increase at $p \leq 0.05$ when compared with the control. At week 5 (Fig. 1b), it significantly increased, alongside another dose group at $p \leq 0.05$ when compared with the control.

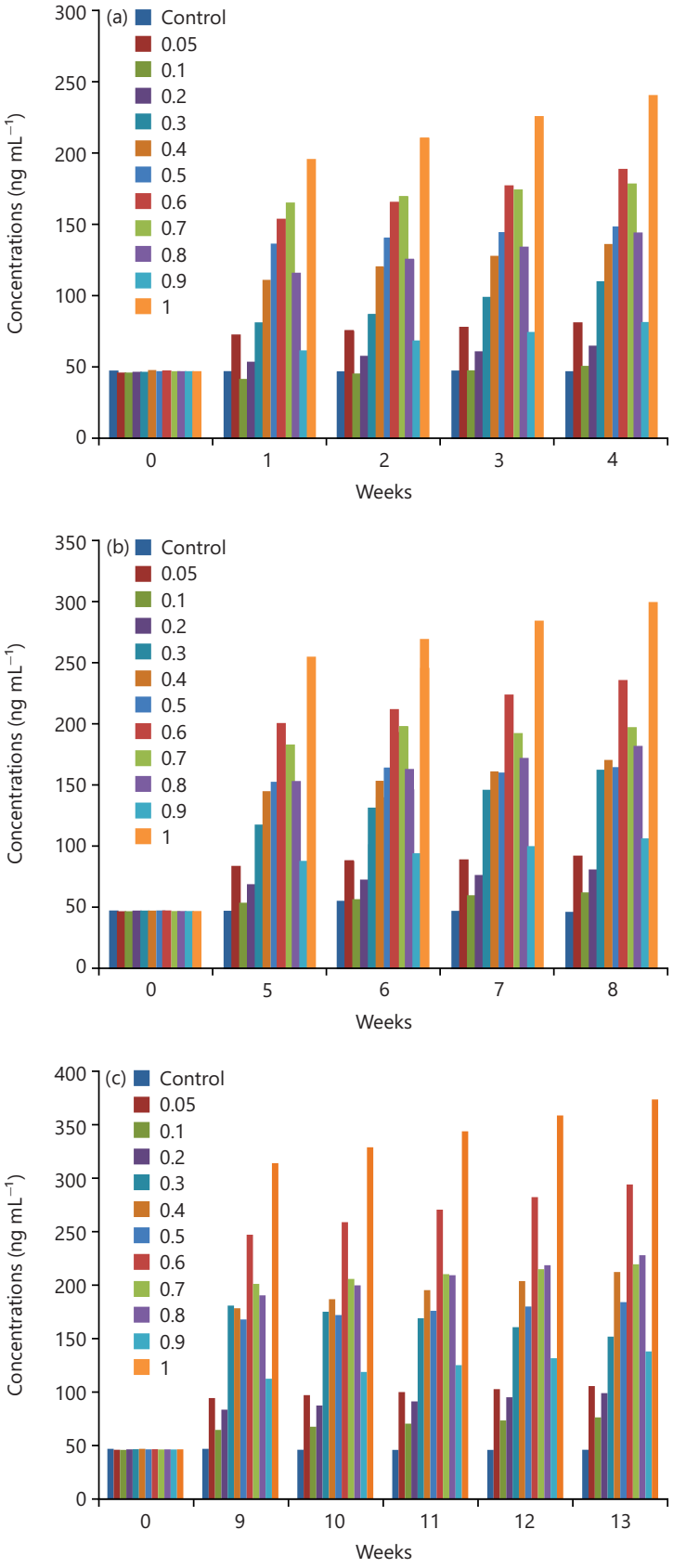

Fig. 1(a-c): Chart of concentration against weeks (durations) for serum luteinizing hormone, (a) Luteinizing hormone concentration after 1 month of BPA administration, that is from 1-4 weeks, (b) Luteinizing hormone concentration following continuous administrations of BPA to the 2nd month (5-8 weeks) and (c) Luteinizing hormone concentration for the 3rd month of BPA administration (9-13 weeks)

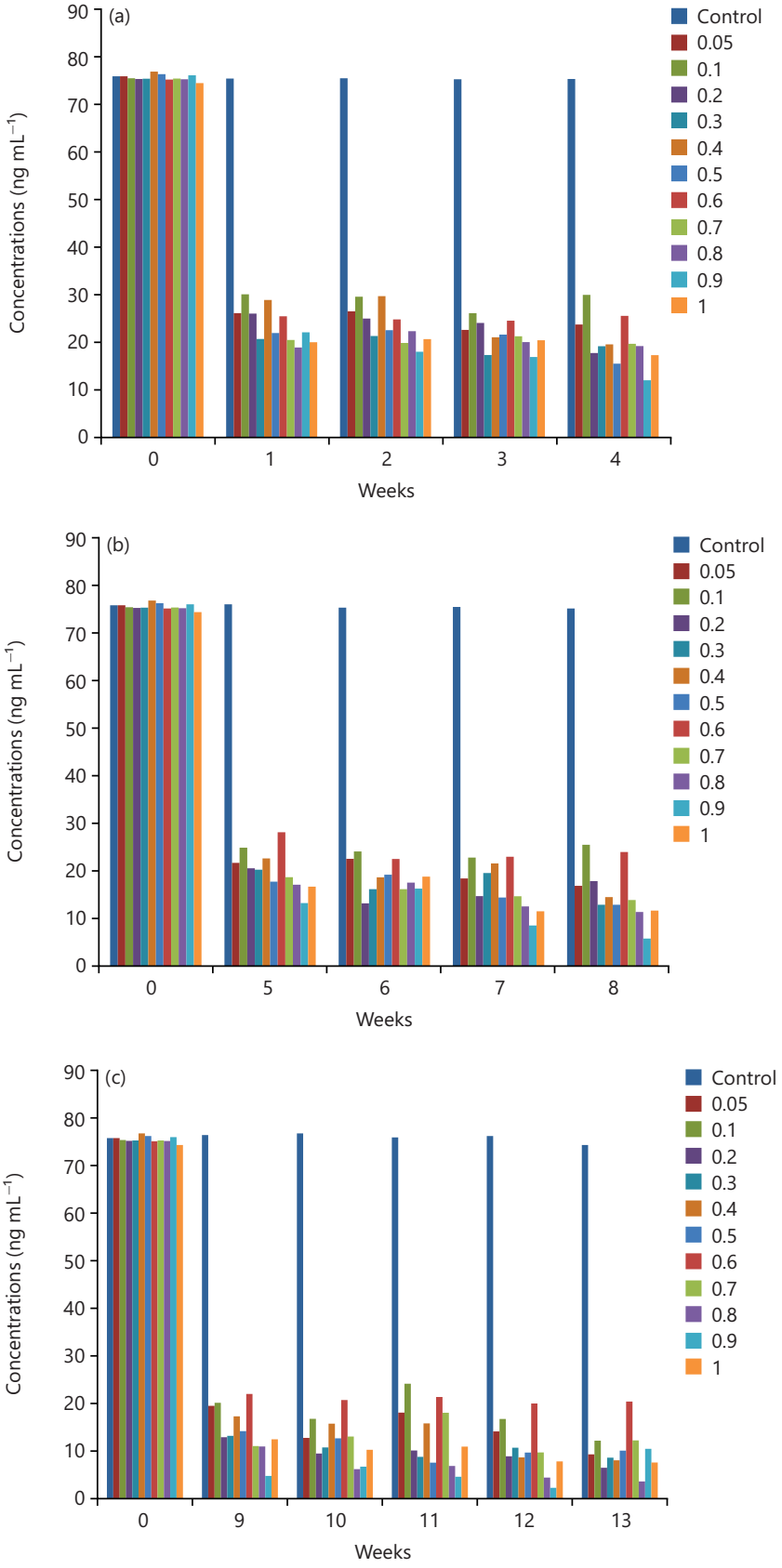

Fig. 2(a-c): Chart of concentration against weeks (durations) for follicle stimulating hormone concentration, (a) Follicle stimulating hormone concentration after 1 month of BPA administration, that is from 1-4 weeks, (b) Follicle stimulating hormone concentration following continuous administrations of BPA to the 2nd month (5-8 weeks) and (c) Follicle-stimulating hormone concentration for the 3rd month of BPA administration (9-13 weeks)

All the dose groups showed a steady and gradual increase over time (Fig.1a-c). At all points in time, the dose group 0.05 mg kg^{-1} rise, then dropped to 0.1 and then there is a dose-dependent increase which peaks at 0.7 mg kg^{-1} for week 1 and 2, for week 3-13, the peak was observed at 0.6 mg kg^{-1}, then from the various peaks established there is a dose-dependent decrease up to 0.9 mg kg^{-1} and finally a spike in the concentration of LH for the dose group 1 mg kg^{-1}, with the highest concentration of LH been observed at week-13 (373.43±0.005) in Fig. 1c and the lowest at week 1 for 1 mg kg^{-1} exposure group (194.93±0.005) in Fig. 1a.

Effect on follicle-stimulating hormone: There is a significant decrease in the follicle-stimulating hormone level of BPA-treated rats when compared with the control at $p \leq 0.05$ (Fig. 2a-c). Although the decrease is steady in course of time, no particular pattern was established except in group 7 (0.6 mg kg^{-1}) (Fig. 2a). There is a characteristic non-steady and non-dose-dependent weekly effect.

Dose groups 0.1, 0.4 and 0.6 mg kg^{-1} consistently exhibited a high concentration of follicle stimulating hormone relative to other dose groups at different times of the experiment. The group exposed to 0.1 mg kg^{-1} showed high FSH levels at weeks 1, 2, 3, 4 (Fig. 2a), 6, 8 (Fig. 2b) and 11 (Fig. 2c), the group exposed to 0.4 mg kg^{-1} showed high FSH levels at weeks 1 and 2, while the 0.6 mg kg^{-1} exposure group showed high FSH level at weeks 5, 7, 9, 10, 12 and 13. (Fig. 2b-c).

DISCUSSION

Luteinizing Hormone (LH) produced in the anterior pituitary gland, is responsible for triggering ovulation and development of the corpus luteum and stimulates specific cells to produce testosterone. LH works synergistically with FSH. Follicle Stimulating Hormone (FSH) released and synthesized by the anterior pituitary gland regulates the body's development, growth, maturation and regulates reproductive processes. FSH signals the follicles in the ovary to begin maturing in preparation for ovulation

The present study examined the effect of BPA exposure on serum luteinizing hormones and follicle-stimulating hormones. It was observed that as luteinizing hormones were increased, FSH was decreased. The ability of BPA to alter responses to sex hormones in both male and female organisms has been investigated. Studies revealed that plasma concentrations of luteinizing hormone were increased[25]. Low-dose BPA decreased the expression of steroidogenic enzymes[26]. Khmiri *et al.*[27] reported increased Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) levels. Hanaoka *et al.*[28] showed decreased FSH levels. A significant inverse association between urine BPA and serum FSH have been reported. Environmentally relevant BPA levels (10-50 µg/kg/day) have adverse effects on hormonal function by decreasing pituitary Luteinizing Hormone (LH) secretion[29]. BPA interfere with Luteinizing Hormone (LH) receptor-ligand binding[30]. In an animal study, BPA resulted in a low FSH and high LH[31]. Multiple studies have shown a relationship between BPA exposure and steroid hormone production[32]. Although epidemiological studies on BPA suggest that both low and high doses of BPA increase corticotropin-releasing hormone levels[33] and luteinizing hormone excess[34]. This indicated that lower dose BPA exposure may produce similar endocrine disrupting effects as suggested by previous studies with the majority of women showing luteinizing hormone excess[34] and BPA impairs follicle growth and induces ovarian abnormalities[35]. Thus, it is likely that high BPA exposure in women may alter the reproductive hormones of a female.

Interestingly, in male species, Studies have reported that BPA exposure is associated with a variety of adverse effects on the reproductive system including decreased steroidogenesis in the testis and decreased serum Follicle-Stimulating Hormone (FSH)[36]. Hanaoka *et al.*[28] reported an inverse correlation between BPA levels and serum FSH concentrations in men. Meeker *et al.*[37] observed urine BPA concentrations were positively associated with the FSH level. Skledar and Mašič[38] found a significant

positive association between BPA and Sex Hormone-Binding Globulin (SHBG) levels. Epidemiological studies have investigated the association between BPA exposure and serum hormone levels in men. Increased BPA exposure, decreased, luteinizing hormone, decreased the ratio of free testosterone to the luteinizing hormone in males[38] and increased serum levels of follicle-stimulating hormone.

This implies that BPA can alter steroid hormone activity and synthesis and in response to sex hormones alteration due to exposure to BPA, BPA can impair implantation and impair the establishment of pregnancies leading to a high rate of infertility.

CONCLUSION

BPA is disruptive to women's hormone homeostasis. The present study is evident that BPA exposure alters the reproductive hormones. As a powerful endocrine disruptor, it affects the activity of pituitary lactotroph cells. BPA may stimulate cell proliferation and stimulates uterine, vaginal and mammary growth and differentiation *in vivo*. The present study has added to the evidence that BPA is also disruptive to women's hormone homeostasis. Collectively, these data indicate that BPA exposure alters sex hormone levels in female species, but strain and species as well as other confounders such as time and route of exposure and age at analysis, may modulate the sensitivity to BPA effects.

SIGNIFICANCE STATEMENT

This study discovers that BPA affects sex hormones and continuous BPA exposure is implicative in a variety of adverse effects on the reproductive system including altered steroidogenesis and altered ovarian steroidogenic gene. This study will help the researcher to uncover the extent of women's hormone homeostasis disruption by BPA. Thus, a new theory on the relationship between BPA exposure, steroid hormone production and fertility may be arrived at.

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