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Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic–pituitary–testicular axis

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ABSTRACT

Reproductive physiology involves complex biological processes that can be disrupted by exposure to environmental contaminants. The effects of bisphenol A (BPA) on spermatogenesis and sperm quality is still unclear. The objective of this study was to investigate the reproductive toxicity of BPA at dosages considered to be safe (5 or 25 mg BPA/kg/day). We assessed multiple sperm parameters, the relative expression of genes involved in the central regulation of the hypothalamic–pituitary–testicular axis, and the serum concentrations of testosterone, estradiol, LH and FSH. BPA exposure reduced sperm production, reserves and transit time. Significant damage to the acrosomes and the plasma membrane with reduced mitochondrial activity and increased levels of defective spermatozoa may have compromised sperm function and caused faster movement through the epididymis. BPA exposure reduced the serum concentrations of testosterone, LH and FSH and increased the concentration of estradiol. The relative gene expression revealed an increase in gonadotropin releasing hormone receptor (*Gnrhr*), luteinizing hormone beta (*Lhb*), follicle stimulating hormone beta (*Fshb*), estrogen receptor beta (*Esr2*) and androgen receptor (*Ar*) transcripts in the pituitary and a reduction in estrogen receptor alpha (*Esr1*) transcripts in the hypothalamus. In this study, we demonstrated for the first time that adult male exposure to BPA caused a reduction in sperm production and specific functional parameters. The corresponding pattern of gene expression is indicative of an attempt by the pituitary to reestablish normal levels of LH, FSH and testosterone serum concentrations. In conclusion, these data suggest that at dosages previously considered nontoxic to reproductive function, BPA compromises the spermatozoa and disrupts the hypothalamic–pituitary–gonadal axis, causing a state of hypogonadotropic hypogonadism.

1. Introduction

BPA is a chemical produced in high volumes and widely used in many consumer products, such as polycarbonate plastics, epoxy resins, PVC, food packaging, dental sealants, and thermal paper receipts. BPA residues were identified in surface water (Belfroid et al., 2002; Rodriguez-Mozaz et al., 2004) and in fish tissues (Belfroid et al., 2002) and may migrate from cans to food (Cao et al., 2010; Grumetto et al., 2008) and from polycarbonate baby bottles to milk (Wong et al., 2005). Thus, humans may be exposed to BPA through different routes, including ingestion, inhalation and dermal exposure.

Reproductive physiology involves complex biological processes that can be disrupted by environmental contaminant exposure. This exposure may be partially responsible for the increase in male reproductive pathologies classified as testicular dysgenesis syndromes, including hypospadias, cryptorchidism, testicular cancer, and low sperm production in adulthood (Lucas et al., 2009).

BPA is a recognized endocrine disruptor with estrogenic activity. The estrogenic activity of BPA is weak when compared with ethinylestradiol or diethylstilbestrol because BPA has a relatively low affinity for nuclear estrogen receptors (Richter et al., 2007). However, its estrogenic potency is equivalent to estradiol

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Table 1
Primers used for real-time PCR analyses.

Gene	NCBI reference sequence	Primer sequence (5′-3′)
Gnrhr (Gonadotropin releasing hormone receptor)	NM_031038.3	F: GCTGCCTGTTCATCATCCCT R: CTGTAGTTTGCGTGGGTCCT
Gnrh1 (Gonadotropin-releasing hormone 1)	NM_012767.2	F: AGGAGCTCTGGAACGTCTGAT R: AGCGTCAATGTCACACTCGG
Fshb (Follicle stimulating hormone, beta polypeptide)	NM_001007597.1	F: AAGTCGATCCAGCTTTGCAT R: GTCCCAGGCCTCTTACAGTG
Lhb (Luteinizing hormone beta)	NM_012858	F: ATGAGTTCTGCCCAGTCTGC R: GTGGGTGGGCATCAGAAGAG
Ar (Androgen receptor)	NM_012502.1	F: GCCATGGGTTGGCGGTCCTT R: AGGTGCCTCATCCTCACGCACT
Esr1 (Estrogen receptor 1, ER alpha)	NM_012689.1	F: CCATATCCGGCACATGAGTA R: TGAAGACGATGAGCATCCAG
Esr2 (Estrogen receptor 2, ER beta)	NM_012754.1	F: CTCACGTCAGGCACATCAGT R: TGTGAGCATTCAGCATCTCC
Ppia (Peptidylprolyl isomerase A)	NM_017101.1	F: GTCAACCCCACCGTGTTCTTC R: ACTTGCCACCAGTGCCATTATG

F: forward; R: reverse.

responses that are mediated by the membrane estrogen receptor GPR30 (Alonso-Magdalena et al., 2012).

The effect of BPA on spermatogenesis remains poorly understood. It is known that BPA interferes with processes related to spermatogenesis, such as androgen production (Akingbemi et al., 2004; Roy et al., 2004) and Sertoli cell activity (Fiorini et al., 2004; Salian et al., 2009); however, the action mechanism through which BPA alters sperm quality has not been well elucidated. During spermatogenesis, cytoplasmic organelles are localized to specific regions of the sperm to perform specialized functions. As a result, the mitochondria are located in the intermediate region and generate energy for the flagellum, thereby promoting sperm movement. The integrity of the sperm membrane also determines the ability of the sperm to fertilize the oocyte. Toxicants can alter the energy metabolism of the intermediate region and decrease spermatic fertility (O'Connell et al., 2002). The oxidative stress induced by toxicants is the most common cause of damage to the sperm (Pasqualotto et al., 2000).

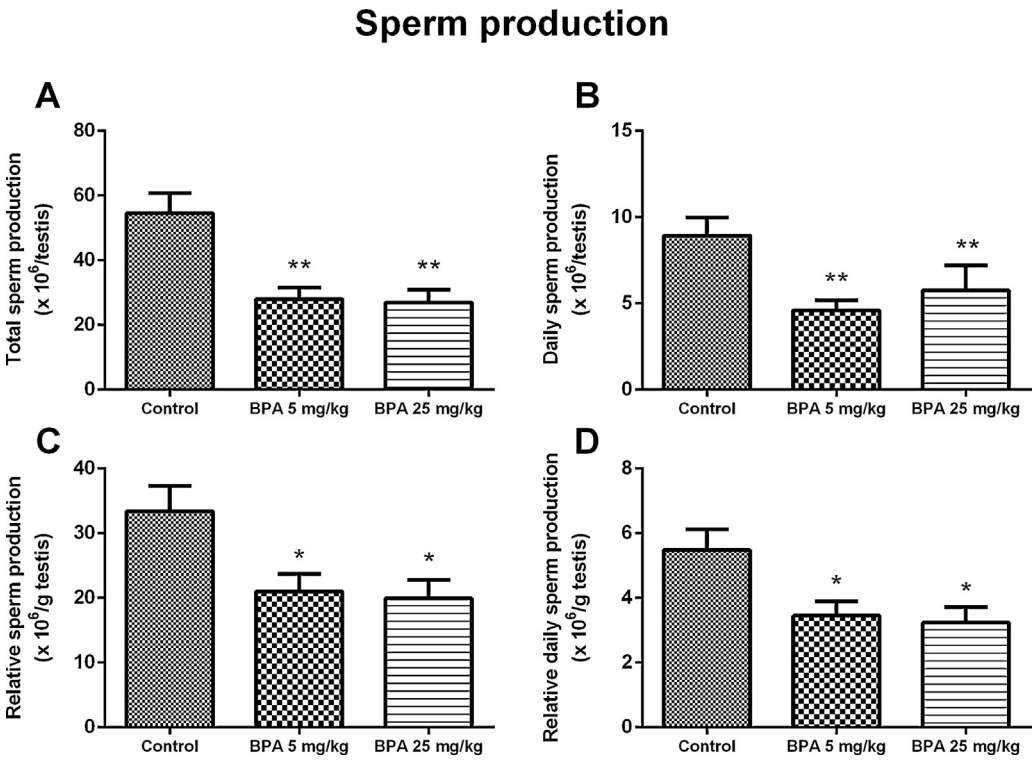

Fig. 1. Total sperm production (A), daily sperm production (B), relative sperm production (C) and relative daily sperm production (D) in rats exposed to bisphenol A (BPA). Data are shown as the mean ± S.E.M., $n = 10$ animals/group, ANOVA followed by Tukey HSD test, $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* control.

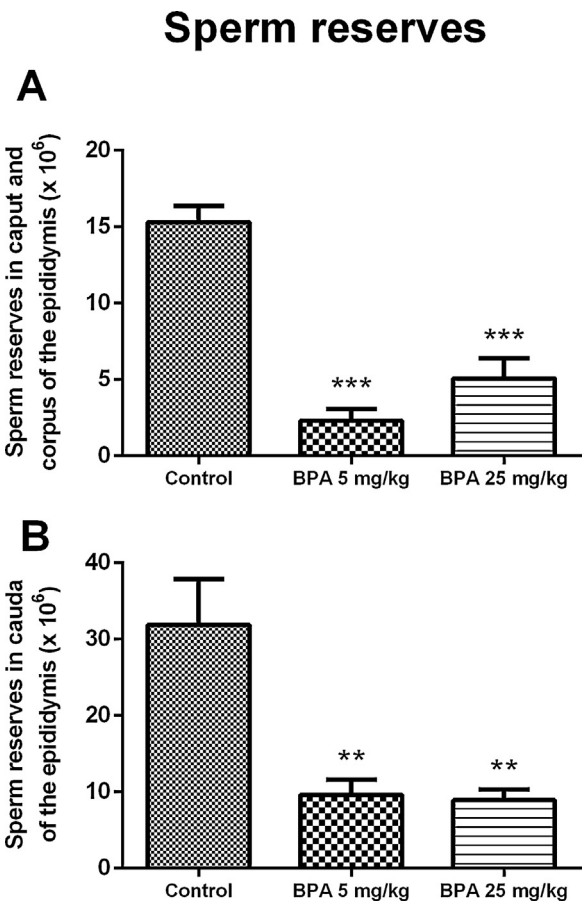

Fig. 2. Sperm reserves in the caput, corpus (A) and cauda (B) epididymis in rats exposed to bisphenol A (BPA). Data are shown as the mean ± S.E.M., $n = 10$ animals/group, ANOVA followed by Tukey HSD test, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* control.

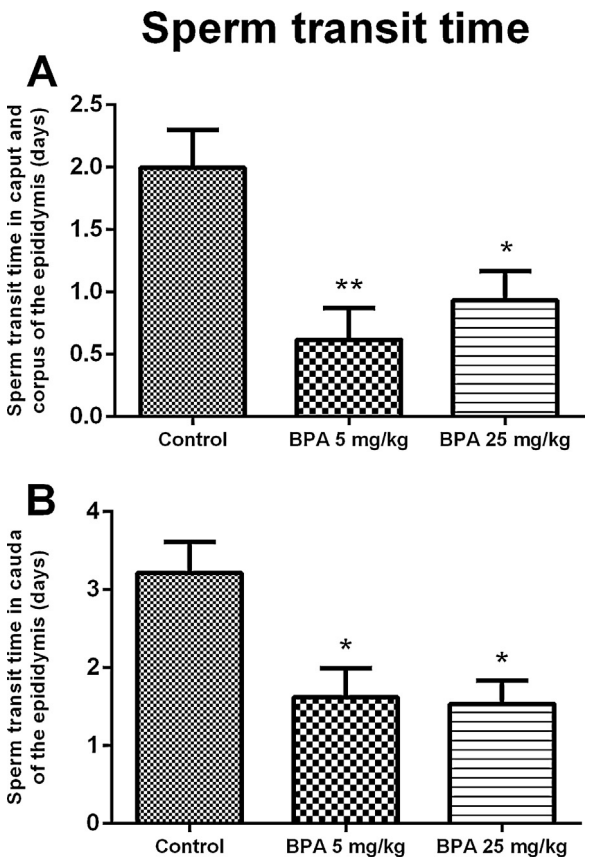

Fig. 3. Sperm transit time through the caput, corpus (A) and cauda (B) epididymis in rats exposed to bisphenol A (BPA). Data are shown as the mean ± S.E.M., $n = 10$ animals/group, ANOVA followed by Tukey HSD test, $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* control.

The objective of this study was to investigate the toxic effects of BPA on the reproductive system at dosages considered to be safe. We assessed sperm production, reserves and transit time, the integrity of the acrosome and plasma membrane, and mitochondrial activity. The effect of BPA on the regulation of the hypothalamic–pituitary–testicular axis was also evaluated by analyzing the relative gene expression of gonadotropin-releasing hormone (*Gnrh1*), androgen receptor (*Ar*), estrogen receptor alpha (*Esr1*) and estrogen receptor beta (*Esr2*) in the hypothalamus, and gonadotropin-releasing hormone receptor (*Gnrhr*), luteinizing hormone beta (*Lhb*), follicle stimulating beta (*Fshb*), *Ar*, *Esr1* and *Esr2* in the pituitary. In addition, we determined the serum concentrations of testosterone, estradiol, LH and FSH in BPA-exposed rats.

2. Material and methods

2.1. Experimental design

Thirty male Wistar rats (*Rattus norvegicus var albinus*) were randomly divided into 3 groups of 10 rats each, receiving daily BPA dosages (CAS 80-05-7, Sigma–Aldrich Co., St. Louis, USA) of 0 (control), 5 or 25 mg/kg body weight (BW) from postnatal day 50 (PND50) through PND90 and they were euthanized at PND105. BPA was diluted in corn oil and administered daily between 7 and 8 a.m. per os (gavage) at a dosing volume of 0.25 mL/100 g BW. The control group received corn oil alone. The dosages of BPA used in this study were based on the previously reported NOAEL (No Observable Adverse Effect Level) for reproductive and developmental toxicity (50 mg/kg BW/day) in rats (FAO/WHO, 2010; Schwetz and Harris, 1993). The rats were maintained on rat chow (Nuvilab CR-1, Nuvital, PR, Brazil) and water ad libitum under a 12:12 h dark/light cycle in a temperature-controlled room (23 ± 1 °C). All procedures were performed in accordance with the Brazilian College of Animal Experimentation and were approved by the Universidade Estadual do Centro-Oeste – Ethical Committee for Animal Research (protocol 057/2012).

2.2. Sperm count

The testes and epididymis (caput, corpus and cauda) were weighed. The tunica albuginea was removed from the testes, and the parenchyma was homogenized in 5 mL saline-Triton 0.5% by sonication for 30 s, 12 kHz (Ultrasonic homogenizer DES500, Unique, Brazil). The samples were then diluted 10 times in saline, and the sperm heads were counted using a hemocytometer. The results were reported as the number of spermatid per testis and per gram of testis. These values were subsequently divided by 6.1 days to calculate the daily sperm production (DSP). The segments of the epididymis (caput, corpus and cauda) were individually minced, homogenized by sonication, diluted and counted as described for the testes. The number of spermatozoa in each homogenate was determined as described above, and the total numbers of spermatozoa for the parts of the epididymis were calculated. The mean time for sperm transit through the epididymis was also calculated by dividing the number of

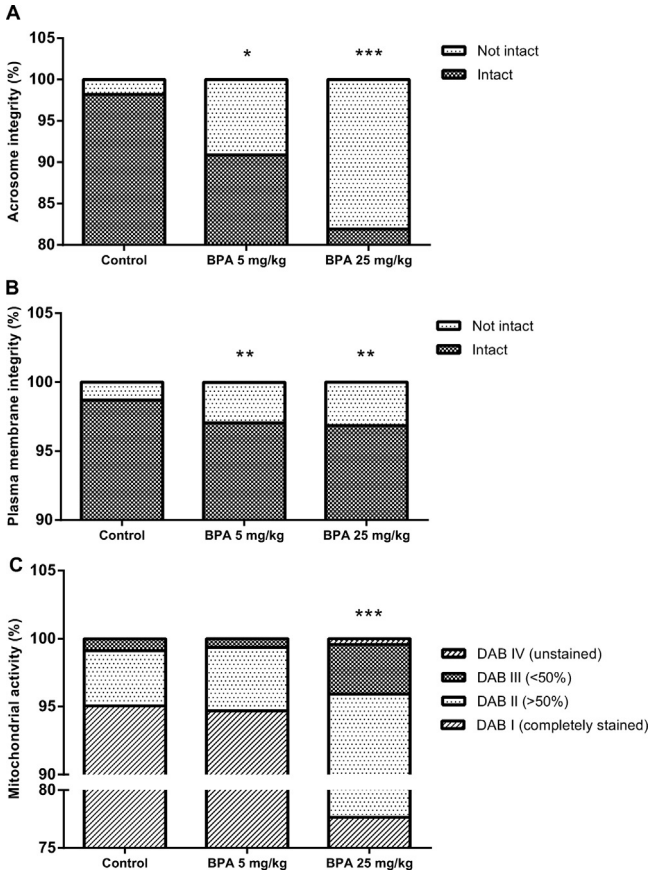

Fig. 4. Sperm parameters in male rats exposed to bisphenol A (BPA). Mean values for the frequency of (A) acrosome integrity, (B) plasma membrane integrity and (C) mitochondrial activity for the control, 5 mg/kg BW and 25 mg/kg BW BPA-exposed groups. Data are shown as the mean ± S.E.M., $n = 10$ animals/group, ANOVA followed by Tukey HSD test, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* control.

spermatozoa in each portion of the epididymis by the DSP of the associated testis.

2.3. Determination of sperm integrity, activity and morphology

2.3.1. Sperm collection

At PND105, the cauda of the epididymis was cut and squeezed, and twenty microliters of pouring fluid was rapidly collected and gently suspended in 200 μL of seminal extender tris–fructose–citric acid (328.8 mM Tris, 91.3 mM fructose, 115.8 mM citric acid, supplemented with 1000 UI penicillin/ml, 1000 μg of streptomycin/mL, 5% glycerol) at 37 °C, as previously described (Mathias et al., 2014). The samples were then immediately evaluated for acrosome integrity, plasma membrane integrity, mitochondrial activity and sperm morphology.

2.3.2. Acrosome integrity

Acrosome integrity was evaluated by a single-stain solution containing 1% (w/v) rose bengal (Sigma–Aldrich Co, MO, USA), 1% (w/v) fast green FCF (Sigma–Aldrich Co, MO, USA), and 40% ethanol in 200 mM disodium phosphate buffer containing 100 mM citric acid at pH 7.2, as previously described (Pope et al., 1991). Five microliters of each sample was incubated with 5 μL of the single-stain solution for 1 min at 37 °C. The total volume (10 μL) was then pipetted onto a slide and smeared with another slide, air-dried at 37 °C and analyzed by light microscopy (1000×). Two hundred spermatozoa were counted per slide and were classified as having an intact or not intact acrosome.

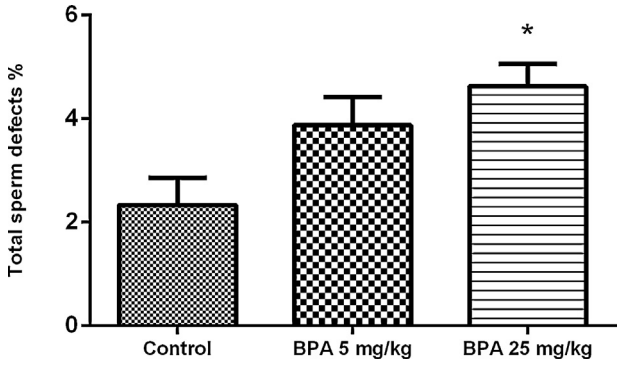

Fig. 5. Frequency of sperm abnormalities in male rats exposed to bisphenol A (BPA). Data are shown as the mean ± S.E.M., $n = 10$ animals/group, ANOVA followed by Tukey HSD test, $^{*}P < 0.05$ *vs.* control.

2.3.3. Plasma membrane integrity

Plasma membrane integrity was evaluated by eosin-nigrosin stain, as previously described (Barth and Oko, 1989). The stain solution was prepared with 1% eosin Y (Sigma–Aldrich Co, MO, USA) and 10% nigrosin (cat number 198285, Sigma–Aldrich Co, MO, USA) in water. Five microliters of each sample was mixed with 5 μL of the stain, and then, the total volume were pipetted onto a slide, smeared with another slide, and air-dried at room temperature. Two hundred spermatozoa were counted per slide by light microscopy (1000×) and were classified as having an intact or not intact membrane.

2.3.4. Mitochondrial activity

Mitochondrial activity was evaluated *via* measurement of cytochrome c oxidase activity in the intermediate piece, as previously described (Hrudka, 1987). The DAB (Sigma–Aldrich Co, MO, USA) was diluted in PBS (2.7 mM KCl, 137 mM NaCl, 8 mM NaHPO4, 1.4 mM KPO4, pH 7.4) to a final concentration of 1 mg/mL and frozen until use. Ten microliters of each sample was incubated with 300 μL of DAB solution pre-heated to 37 °C for 1 h at 37 °C in the dark. Ten microliters of each sample was pipetted onto a slide, smeared with another slide, and air-dried at 37 °C in the dark. The slide samples were then incubated for 10 min in a fixative solution containing 10% formaldehyde (Sigma–Aldrich Co, MO, USA) in PBS and allowed to air-dry at 37 °C in the dark. Two hundred spermatozoa from each sample were analyzed by phase-contrast microscopy at a magnification of 400× and then classified based on the degree of staining of the intermediate piece as follows: DAB I (completely stained), DAB II (>50%), DAB III (<50%) and DAB IV (unstained).

2.3.5. Sperm morphology

The samples were diluted by a factor of 10 in a buffered formal-saline solution [34.72 mM Na2HPO4·2H2O, 18.68 mM KH2PO4, 92.4 mM NaCl, 12.5% (v/v) formaldehyde] and examined as wet preparations by phase contrast microscopy at 400× magnification.

2.4. Hormone measurements

Blood was collected *via* cardiac puncture and centrifuged at 3500 rpm (Excelsa II 206 BL, Sao Paulo, SP, Brazil) for 15 min. The serum was frozen and stored at −70 °C for subsequent hormone evaluation. The serum FSH and LH concentrations were determined by a chemiluminescent immunoassay using Luminex xMAP technology (Milliplex MAP rat pituitary panel, Billerica, MA, USA). The serum testosterone and estradiol concentrations were measured by electrochemiluminescence using commercially

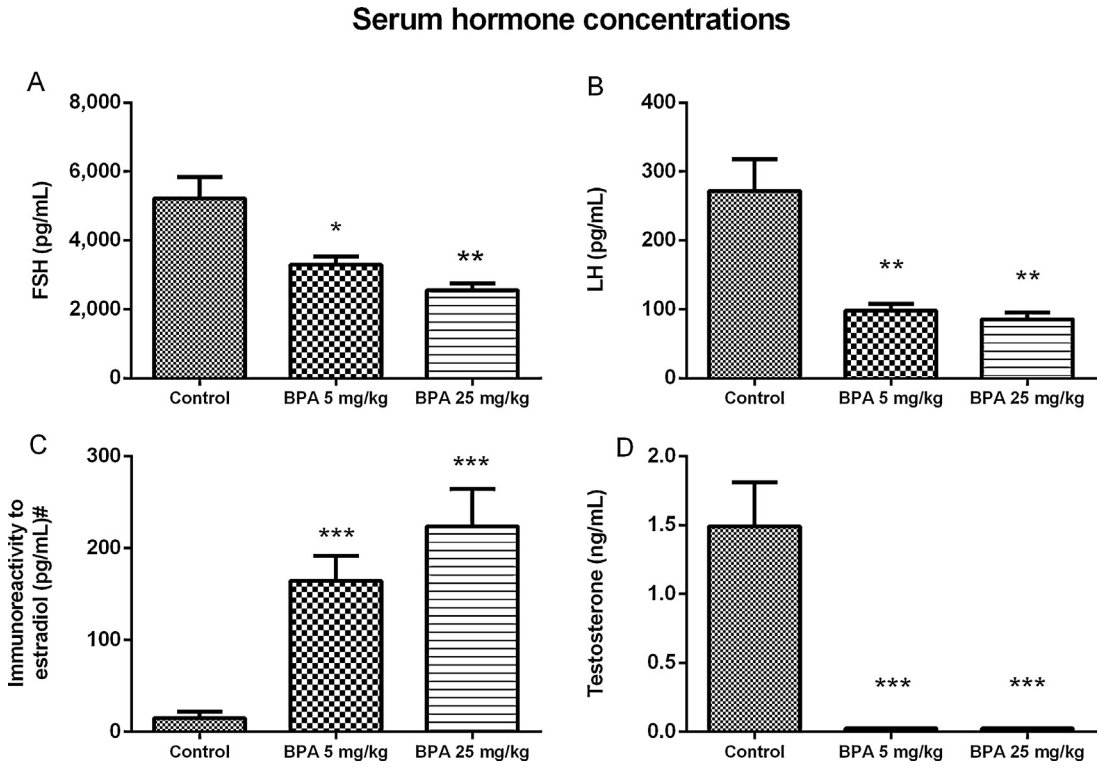

Fig. 6. Serum hormone concentrations in male rats exposed to bisphenol A (BPA). Data are shown as the mean ± S.E.M. for (A) FSH, (B) LH, (C) estradiol and (D) testosterone serum concentrations for the control, 5 mg/kg BW and 25 mg/kg BW BPA-exposed groups. $n = 10$ animals/group, ANOVA followed by Tukey HSD test, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* control, #cross-reactivity with BPA > 62%.

available kits (Elecsys Testosterone II and Estradiol II Immunoassay kits, Roche Diagnostics, Indianapolis, IN, USA). The cross-reactivity between BPA and estradiol was evaluated using a calibration curve (0, 10, 100, 1000 and 10,000 pg/mL of BPA) and measured with the same commercial kit.

2.5. Real-time PCR

Following sacrifice, the pituitary gland and hypothalamus were rapidly removed, frozen in liquid nitrogen and maintained at −70 °C until use. Total RNA was isolated from the hypothalamus and pituitary gland using TRIzol reagent (Lifetechnologies, CA, USA) as previously described (Kizys et al., 2012). The RNA pellet was air-dried and suspended in 30 μL of RNase-free water. The absorbance and concentration were estimated by spectrophotometry with Nanovue Plus (GE Healthcare, UK). Two μg of total RNA recovered from each tissue were used to perform the reverse transcription reaction using MML-V Reverse Transcriptase kit (Promega, Madison, WC) according to the manufacturer's recommended protocol. Real-time PCR was carried out in duplicate for the *Gnrh1*, *Gnrhr*, *Lhb Fshb*, *Ar*, *Esr1* and *Esr2* genes in a 12-μL reaction volume of 2 μL cDNA, 6.5 μL SYBR Green Master Mix (Applied Biosystems), and 10 pmol/L of each primer. The *Ppia* gene was used as an endogenous control. The primer sequences are shown in Table 1.The fluorescence intensity was quantified, and amplification plots were analyzed by a sequence detector system (ABI Prism 7500; Applied Biosystems). The amplification conditions consisted of initial denaturation (95° for 10 min) followed by 45 cycles of 95 °C for 20 s and 58 °C for 20 s (annealing and extension). Quantification was performed by the delta–delta Ct method as described previously (Livak and Schmittgen, 2001).

2.6. Statistical analysis

The variables in question were first submitted to Kolmogorov–Smirnov tests for normality and the Bartlett test for homoscedasticity. The parameters were analyzed by ANOVA test followed by the Tukey HSD (honest significance difference) test. All analyses were performed with Statistica 7.0 (Statsoft Inc., Tulsa, OK, USA). Statistical differences were considered significant when the value of P was lower than 0.05. The values were expressed as means and the standard error of the mean (±SEM).

3. Results

3.1. BPA decreased sperm production, sperm reserves and sperm transit time

BPA exposure during adulthood: (a) reduced the total and daily sperm production by 50% at dosages of 5 mg/kg and 25 mg/kg (Fig. 1); (b) reduced the sperm reserves in all segments of the epididymis for all BPA-treated groups by at least 70% (Fig. 2); and (c) reduced the sperm transit time in the caput, corpus and cauda epididymis by 50% (Fig. 3).

3.2. BPA affects the integrity of the acrosome and plasma membrane, mitochondrial activity and sperm morphology

The integrity of the acrosome was decreased by 8 and 16% in the 5 mg BPA/kg and 25 mg BPA/kg treatment groups, respectively (Fig. 4A). The integrity of the plasma membrane was decreased by 2% in both BPA-treated groups (Fig. 4B). The mitochondrial activity was reduced in the rats that received the highest dosage of BPA, and the sum of the DAB score alterations was 19% (Fig. 4C). BPA

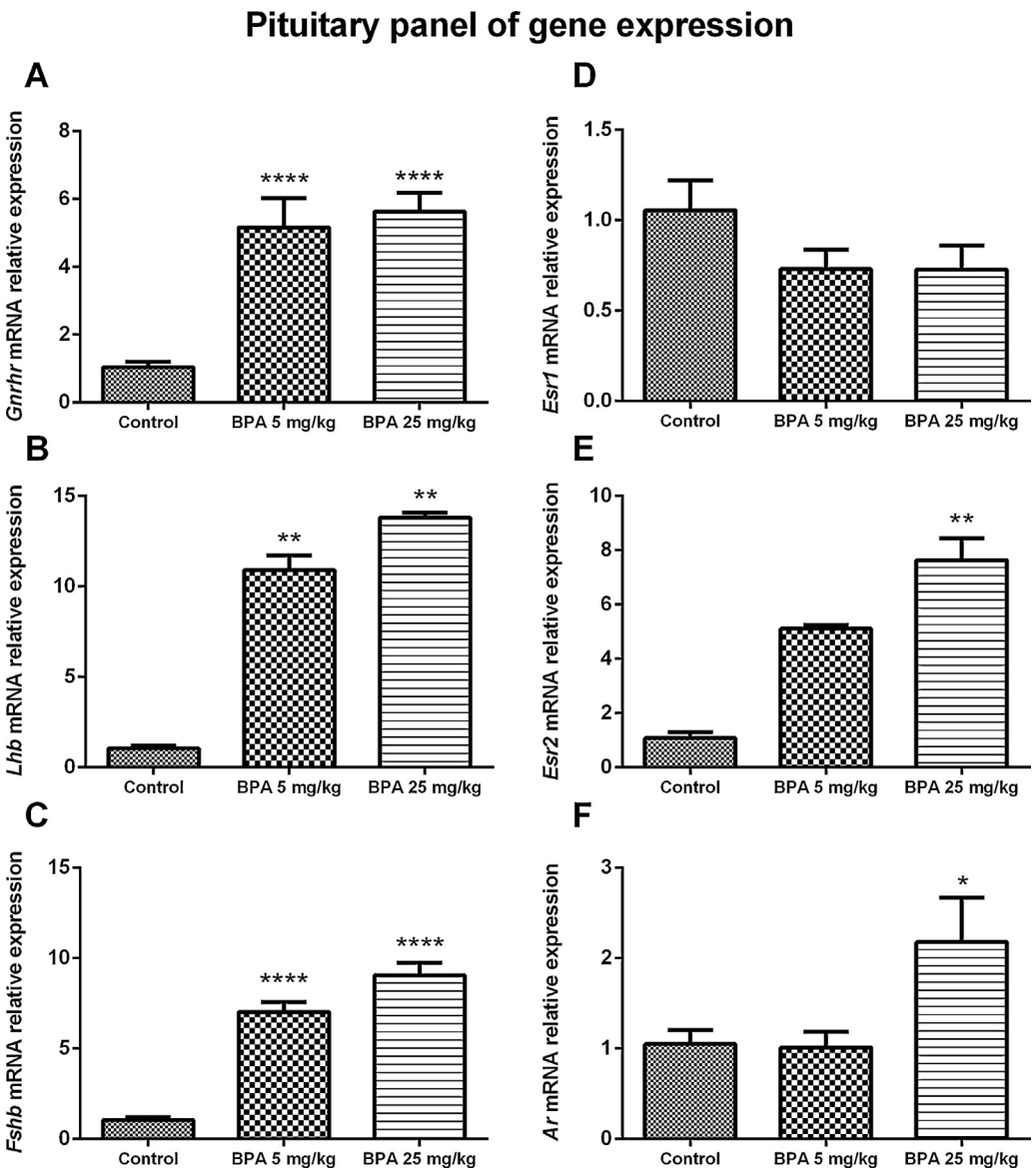

Fig. 7. The pituitary panel of relative gene expression in male rats exposed to bisphenol A (BPA). mRNA data are shown as the mean ± S.E.M. for (A) *Gnrhr*, (B) *Lhb*, (C) *Fshb*, (D) *Esr1*, (E) *Esr2* and (F) *Ar* for the control, 5 mg/kg BW and 25 mg/kg BW BPA-exposed groups. $n = 10$ animals/group, ANOVA followed by Tukey HSD test, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{****}P < 0.0001$ *vs.* control.

exposure increased the number of abnormalities observed in the spermatic cells of rats that were exposed to the highest dosage of BPA (Fig. 5).

3.3. BPA disrupts serum hormone concentrations

In both groups exposed to BPA, all hormones measured were altered: serum concentrations of FSH were reduced by 38% and 52% in dosages of 5 mg and 25 mg BPA/kg, respectively (Fig. 6A); serum concentrations of LH were reduced by 65% and 70%, respectively (Fig. 6B); and serum concentrations of testosterone were reduced by 98% for both BPA-treated groups (Fig. 6D). The immunoreactivity for estradiol in the serum was 100 times greater in 5 mg/kg and 150 times in 25 mg/kg (Fig. 6C); however, nonlinear BPA cross-reactivity, independent of BPA concentration was identified in this assay at levels greater than 62%.

3.4. BPA modulates the expression of genes involved in the hypothalamic–pituitary–testicular axis

In the pituitary, the relative levels of *Gnrhr* mRNA were increased five-fold in both BPA-exposed groups (Fig. 7A). The relative levels of *Lhb* mRNA were increased 10-fold in 5 mg and 13-fold in 25 mg BPA/kg (Fig. 7B). The relative levels of *Fshb* mRNA were increased 6-fold in 5 mg and 8-fold in 25 mg BPA/kg (Fig. 7C). Respectively, the relative levels of *Esr2* and *Ar* were increased seven and two-fold in rats exposed to 25 mg BPA/kg BW (Fig. 7E and F). The expression of *Esr1* was not affected by BPA exposure.

In the hypothalamus, the expression of *Gnrh* was approximately 50% decreased in the low dosage BPA group and 70% in the high dosage BPA group. However, no statistically significant differences were observed (Fig. 8A). The expression of *Esr1* was reduced 50% in the 5 mg BPA/kg BW treatment group (Fig. 8C). The expression of *Ar* and *Esr2* were not affected by BPA exposure (Fig. 8B and D).

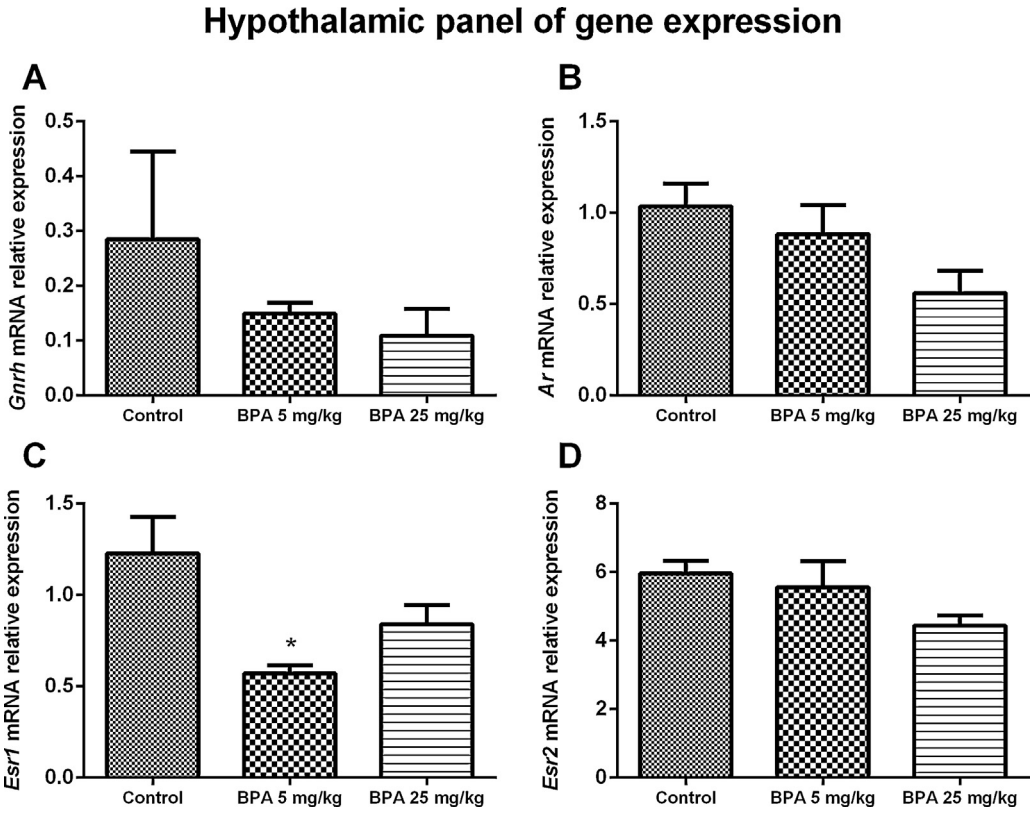

Fig. 8. The hypothalamic panel of relative gene expression in male rats exposed to bisphenol A (BPA). mRNA data are shown as the mean ± S.E.M. for (A) *Gnrh*, (B) *Ar*, (C) *Esr1* and (D) *Esr1* for the control, 5 mg/kg BW and 25 mg/kg BW BPA-exposed groups. $n = 10$ animals/group, ANOVA followed by Tukey HSD test, $^{*}P < 0.05$ *vs.* control.

4. Discussion

BPA, an estrogenic endocrine disruptor, has been shown to impair male fertility but its effect on spermatogenesis is poorly understood. BPA interferes with processes related to spermatogenesis, such as androgen production (Akingbemi et al., 2004; Roy et al., 2004) and Sertoli cell activity (Fiorini et al., 2004; Salian et al., 2009), but shows a weak affinity for estrogen receptors alpha (ESR1) and beta (ESR2) (Welshons et al., 2003). Therefore, we investigated the reproductive toxicity of BPA using adult male Wistar rats as an experimental model. Rats were exposed to dosages corresponding to half (25 mg/kg BW/day) and ten-fold lower than (5 mg/kg BW/day) the NOAEL for reproductive and developmental toxicity (50 mg/kg BW/day) (FAO/WHO, 2010; Schwetz and Harris, 1993).

Results showed that BPA exposure in adulthood reduced the total and daily testicular sperm production by approximately 50% in rats from both BPA groups. The sperm reserves in the epididymis were also compromised, and a reduction of approximately 75% was observed in the caput and cauda with no differences between the dosages observed. In mice, gestational exposure to 0.020 mg/kg BPA affected the sperm production in offspring (Vom Saal et al., 1998). As reported by Salian et al. (2011), exposure to BPA in rats during the prenatal and neonatal period also affects testicular development and spermatogenesis in adult offspring.

Nevertheless, results from experiments conducted in adult animals are contradictory. Sakaue et al. (2001) demonstrated that BPA exposure in adults exposed to 0.02 mg/kg for 6 days compromises the daily sperm production, preventing its normal increase from 14 to 18 weeks. Curiously, when another group repeated this experiment, no alterations were found (Ashby et al., 2003). However, in the same year Chitra et al. (2003) showed that 45 day-old male rats submitted to BPA treatment at dosages of 0.0002, 0.002 or 0.02 mg/kg presented a dose-dependent reduction in sperm motility and epididymal sperm count. Recently, Liu et al. (2013) also reported a decreased sperm count after long-term exposure to 0.2 mg/kg BPA in rats, and Kourouma et al. (2014) observed a reduction in epididymal sperm motility and count in a dose dependent manner for the 10 and 50 mg/kg treatment groups. These experiments showed a lowest observed adverse effect level (LOAEL) varying from 0.0002 mg/kg to 10 mg/kg and in some case a dose-dependent effect, which was not observed in this study. Differences in these LOAELs may be related to the duration of treatment, administration route and the age of the animals at the beginning of the studies.

In humans, a prospective study in couples undergoing medically assisted reproduction identified BPA residues in 98% of the patient urine samples, and the amount of BPA was inversely correlated with sperm concentration and motility (Knez et al., 2014). However, humans are exposed to a cocktail of environmental toxicants, where the toxicity of each may be altered in these mixtures (Kortenkamp, 2014). Thus, BPA exposure is likely not solely responsible for modifications to human sperm parameters.

The reduction in sperm production could be associated with the disruption of the spermatic cycle. In adulthood, BPA exposure decreases sperm count *via* the reduction in type A spermatogonial, spermatocytes and spermatids (Jin et al., 2013). Inhibition of spermiation, characterized by an increase in stage VII and a decrease in stage VIII in the seminiferous epithelium, was also described (Liu et al., 2013). Increased apoptosis of spermatic and Sertoli cells was also observed (Wang et al., 2014).

The sperm transit time through the caput and cauda of the epididymis was reduced in the BPA-treated groups. This faster transit of the spermatozoa across the epididymal segments may compromise its maturation process because important events occur during this period of time (Robaire et al., 2006).

Fertility can be evaluated by sperm concentration, motility and morphology (Ford, 2010; Menkveld, 2010; WHO, 1999). However, methods that assess the functional competence of sperm more accurately indicate subfertility and infertility (Aitken, 2006). Measures of sperm functionality reflect its ability to fertilize the oocyte because higher levels of membrane integrity and mitochondrial activity correspond to an enhanced interaction of the sperm with the female gamete, improving the fertilization process *in vivo* or *in vitro* (Aitken, 2006; Rijsselaere et al., 2005; Rodriguez-Martinez, 2003).

In addition to a decrease in sperm production and reserves, the rapid movement of the sperm through the epididymis was observed in BPA-exposed rats, which may have compromised its function. Toxicants can alter the energy metabolism of the intermediate piece and decrease spermatic fertility (O'Connell et al., 2002). The resulting oxidative stress due to the lower expression of antioxidants (Vernet et al., 2004) is the most common cause of damage to the sperm (Pasqualotto et al., 2000). BPA exposure is related to a decrease in the activity of the antioxidant system, causing an increase in the generation of ROS (Chitra et al., 2003; Kourouma et al., 2014). In the current study, BPA-treated animals showed higher levels of damage in the acrosomes and plasma membrane, a reduction in mitochondrial activity and increased levels of defective spermatozoa.

Spermatogenesis is dependent on a well-orchestrated hormonal environment. Leydig cells stimulated by LH provide the local production of testosterone, and Sertoli cells stimulated by FSH provide the local production of estradiol. In addition, Sertoli cells maintain the spermatogonial stem cells responsible for the continuity of spermatogenesis (O'Shaughnessy, 2014). In the present study, BPA exposure caused an imbalance in these hormones, which may have contributed to defects in spermatogenesis and sperm maturation. Using prepubertal rats as an experimental model, Nakamura et al. (2010) also reported a dose-dependent reduction in testosterone and LH serum concentrations (20, 100 and 200 mg BPA/kg/day).

The reduction in the LH serum concentration may be directly responsible for the reduction in testosterone production by Leydig cells in BPA-treated animals. In the seminiferous tubules, testosterone is carried by androgen binding protein (ABP) through the testis toward the epididymis. Testosterone is converted to dihydrotestosterone (DHT) by 5-alpha-reductase enzyme (Robaire and Hamzeh, 2011; Robaire et al., 2006). The androgenic activity of DHT is two-fold higher than testosterone, and the epididymis is highly dependent on androgens to complete its transport and storage of spermatozoa prior to ejaculation (Robaire and Hamzeh, 2011; Robaire et al., 2006). Therefore, the reduction in testosterone observed in the BPA-treated animals can affect these processes and may be at least partly responsible for the alterations observed in the spermatozoa of these rats.

In addition to its action in the epididymis, testosterone is responsible for the initiation of spermatogenesis and the maintenance of certain Sertoli functions after its conversion to estradiol by aromatase (O'Donnell et al., 2006). Small amounts of estradiol are vital for Sertoli cell activity after binding to estrogen receptors located in the nucleus and cytoplasm (ESR1 and ESR2) or in the plasma membrane (GPER) (Chimento et al., 2014). Conversely, high levels of estradiol compromise sperm production (Pinto et al., 2008). The estrogenic activity of BPA is weak when compared with ethinylestradiol or diethylstilbestrol because BPA has a lower affinity for estrogen receptors (ESR1 and ESR2) (Richter et al., 2007). However, the estrogenic potency of BPA is equivalent to estradiol with regard to responses mediated by the membrane estrogen receptor GPER (Alonso-Magdalena et al., 2012). Activation of ESR1 and GPER culminates with spermatogonial cell proliferation (Chimento et al., 2014); thus, the interaction of BPA with these receptors may trigger signaling pathways in Sertoli cells.

We assessed the effect of BPA on the hormonal regulation of the hypothalamic–pituitary–gonadal axis. Despite increased *Lhb* transcript production in the pituitary of BPA-exposed rats, BPA exposure reduced testosterone and LH serum concentrations. These changes produce a state of acquired hypogonadotropic hypogonadism (Salenave et al., 2012). GnRH receptor expression was increased, which may correspond with the increased testosterone serum concentration. However, the increased expression of androgen and beta-estradiol receptors suggests that negative feedback mechanisms were stimulated (Jeong and Kaiser, 2006). With the exception of a reduction in alpha-estradiol receptor (*Esr1*) expression in rats exposed to the low dosage of BPA, no changes in the expression of the receptors that regulate the HPG axis were observed in the hypothalamus. Studies in *Esr1* knock-out mice showed that the aromatization of testosterone and *Esr1* receptor activation are the predominant mechanisms involved in the estradiol-mediated suppression of GnRH synthesis/release (Lindzey et al., 1998). Our current data suggest that the hypothalamus does not participate in the disruption process caused by BPA exposure.

5. Conclusion

In the present study, we demonstrated for the first time that adult male exposure to BPA caused a reduction in sperm production that was accompanied by a decrease in sperm reserves and sperm transit time. In addition, specific functional parameters were affected, including the compromised integrity of the acrosome and plasma membrane and a reduction in mitochondrial activity. The observed pattern of gene expression is indicative of an attempt by the pituitary to reestablish normal levels of LH, FSH and testosterone serum concentrations. Overall, these data suggest that even at a dosage considered lacking reproductive toxicity, BPA exposure compromises sperm production and functionality and disrupts the hypothalamic–pituitary–gonadal axis resulting in a state of hypogonadotropic hypogonadism.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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