Environmental Pollution xxx (2019) xxx-xxx

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com

Male exposure to bisphenol a impairs spermatogenesis and triggers histone hyperacetylation in zebrafish testes☆

S. González-Rojo, M. Lombó, C. Fernández-Díez, M.P. Herráez*

Department of Molecular Biology, Faculty of Biology, Universidad de León, Campus de Veganaza s/n, León, 24071, Spain

ARTICLE INFO

Article history:
Received 25 July 2018
Received in revised form 16 January 2019
Accepted 17 January 2019
Available online xxx

Keywords:
Bisphenol A
Histone acetylation
DNA methylation
Zebrafish testes
Sperm transcripts
GPER

ABSTRACT

Bisphenol A (BPA) is an endocrine disruptor whose ubiquitous presence in the environment has been related with impairment of male reproduction. BPA can cause both transcriptomic and epigenetic changes during spermatogenesis. To evaluate the potential effects of male exposure to BPA, adult zebrafish males were exposed during spermatogenesis to doses of 100 and 2000 μg/L, which were reported in contaminated water bodies and higher than those allowed for human consumption. Fertilization capacity and survival at hatching were analysed after mating with untreated females. Spermatogenic progress was analysed through a morphometrical study of testes and apoptosis was evaluated by TUNEL assay. Testicular gene expression was evaluated by RT-qPCR and epigenetics by using ELISA and immunocytochemistry. *In vitro* studies were performed to investigate the role of Gper. Chromatin fragmentation and the presence of transcripts were also evaluated in ejaculated sperm. Results on testes from males treated with the highest dose showed a significant decrease in spermatocytes, an increase in apoptosis, a downregulation of *ccnb1* and *sycp3*, all of which point to an alteration of spermatogenesis and to meiotic arrest and an upregulation of *gper1* and *esrrga* receptors. Additionally, BPA at 2000 μg/L caused missregulation of epigenetic remodelling enzymes transcripts in testes and promoted DNA hypermethylation and H3K27me3 demethylation. BPA also triggered an increase in histone acetyltransferase activity, which led to hyperacetylation of histones (H3K9ac, H3K14ac, H4K12ac). *In vitro* reversion of histone acetylation changes using a specific GPER antagonist, G-36, suggested this receptor as mediator of histone hyperacetylation. Males treated with the lower dose only showed an increase in some histone acetylation marks (H3K14ac, H4K12ac) but their progeny displayed very limited survival at hatching, revealing the deleterious effects of unbalanced paternal epigenetic information. Furthermore, the highest dose of BPA led to chromatin fragmentation, promoting direct reproductive effects, which are incompatible with embryo development.

1. Introduction

In recent times, scientific interest in endocrine disruptor molecules, which are capable of interacting with endocrine receptors, has increased considerably. Bisphenol A (BPA, 2,2-Bis(4-hydroxyphenyl)propane) is a well-known environmental endocrine disruptor, widely used in polycarbonate manufacturing and epoxy resins (Rubin, 2011; for review see Manfo et al., 2014) which has become a part of everyday items such as baby bottles, food packaging, dental materials, thermal paper, electronic equipment or medical devices (Shi et al., 2017). Its ubiquitous presence in the environment has resulted in chronic low-level exposure in humans and reported to be found in the urine of approximately 90% of people in the United States (Calafat et al., 2008). The presence of high levels of BPA has been related in particular to health disorders such as diabetes, obesity, cardiovascular diseases or carcinogenesis (Adoamnei et al., 2018; Bansal et al., 2017; Fénichel and Chevalier, 2017; Lombó et al., 2015; Manfo et al., 2014; Rubin, 2011). BPA has also been described as impairing fertility, and as being related to recurrent miscarriages or premature births in women (Rubin, 2011) or correlated to a decrease in seminal quality and DNA damage in spermatozoa in men (Li et al., 2011, 2010). Effects on sperm quality entail low sperm counts, reduced volume and density and poor mobility in mammals (Li et al., 2011; Manfo et al., 2014) and fish (Hatef et al., 2012; Haubruge et al., 2000; Lahnsteiner et al., 2005).

The reported effects are related to the ability of BPA to mimic estrogen actions binding to estrogen receptors (ERs), and promoting genomic (on gene expression) and non-genomic (interference with cell signalling) effects (Acconcia et al., 2015; Manfo et al., 2014). Apart from canonical ERs, estrogen-related receptors (ERRs), particularly ERRγ (Acconcia et al., 2015; Okada et al., 2007) and the seven transmembrane G protein-coupled estrogen receptor, GPER (Prossnitz et al., 2008), show BPA binding affinity. Moreover, according to MacKay and Avizaid (MacKay and Abizaid, 2018), BPA seems to act antagonistically on the androgen receptor (AR), and their anti-androgenic effects have also been claimed to be responsible for BPA male reproductive toxicity (Wang et al., 2017b).

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author.
Email address: paz.herraez@unileon.es (M.P. Herráez)

https://doi.org/10.1016/j.envpol.2019.01.127
0269-7491/

BPA interference with the signalization mediated by these receptors could explain different alterations on male reproductive capacity, such as the reduction of sperm quality, given their interference with cell proliferation or apoptosis during spermatogenesis. Additionally, as has been reported in mice (Berger et al., 2016; Drobná et al., 2018; Xin et al., 2015) and zebrafish (Dong et al., 2018; Lombó et al., 2015), some of the toxic effects of BPA can be transmitted to the next generations via parental inheritance; specifically, paternal exposure increases the percentage of malformed descendants who have never been exposed to BPA. These data imply that other types of changes occur, which are still not well understood and which alter the information provided to the zygote by gametes. Our aim is to evaluate the changes caused by BPA exposure during spermatogenesis in the paternal information, which may be responsible for the transmission of negative effects to the progeny, as observed in zebrafish (Lombó et al., 2015).

As reviewed by Herráez and coworkers (Herráez et al., 2017), paternal information transmitted by sperm is comprised in the genome and also in the epigenetic marks (such as DNA methylation and histone modifications), the non-coding RNAs and the mRNAs, which are transmitted to the zygote upon fertilization, and which could be translated by the embryo in the early stages of development (Swann and Lai, 2016). Alteration of transcriptome or epigenetics during spermatogenesis could be responsible for transgenerational inheritance of the toxic effects of BPA to non-exposed progeny (Lombó et al., 2015). Despite erasure of epigenetic marks of parental-contributed genomes in the early embryo and the establishment of the somatic pattern, considerable evidence confirms that some marks evade the reprogramming process, allowing the paternal transmission of some epigenetic information to the embryo (Brykczynska et al., 2010; Yamauchi et al., 2011). As has been demonstrated, alteration of specific epigenetic marks of mature spermatozoa, such as the repressive histone modification H3K9me3 in *Caenorhabditis elegans* (Klosin et al., 2017) or the reduced methylation at lysine 4 and 9 of histone 3 (H3K4 and H3K9) in mice, can further affect forthcoming progeny (Pérez-Cerezales et al., 2017). Moreover, sperm DNA methylome has been shown to be inherited by embryos in zebrafish (Jiang et al.,, 2013). Exposure to BPA during spermatogenesis may affect the following factors: i) the genome may be affected by chromatin fragmentation as has been reported in somatic human cells (Herz et al., 2017), during rat spermatogenesis (Liu et al., 2014a,b) and in human spermatozoa (Meeker et al., 2010a; 2010b); ii) the transcriptome may be affected by endocrine disruption of estrogenic pathways, and iii) the epigenome may also be affected by BPA, as has lately been reported in several studies (Bhandari et al., 2015; Chianese et al., 2017; Mao et al., 2015; Martin and Fry, 2018; Zheng et al., 2017). Due to the high transcriptional and epigenetic activity required to progress through mitotic and meiotic phases and to achieve the required epigenetic reprogramming, spermatogenesis represents a potential window of susceptibility to BPA exposure.

Our hypothesis is that BPA can affect sperm chromatin integrity and cause both transcriptomic and epigenetic changes during spermatogenesis, which could modify the germ line and potentially alter offspring developmental ability. In order to prove this hypothesis, we used zebrafish (*Danio rerio*) to explore the effects of BPA in the aquatic environment during spermatogenesis.

2. Material and methods

2.1. Chemicals

The toxic 2,2-Bis(4-hydroxyphenyl)propane (BPA) was purchased from Sigma-Aldrich (St. Louis, MO) and the specific GPER antagonist (G36) was obtained from Cayman Chemical (USA). All the antibodies used were acquired from Abcam (Cambridge, UK). All other reagents used in this study were purchased from Sigma-Aldrich, unless noted otherwise.

2.2. Animals

Mature zebrafish (*Danio rerio*) AB strain (6–12 months old) were maintained at 27–29 °C with 14 h light/10 h dark cycle photoperiod in a recirculating water system (pH 7.0–7.5; 400–500 μS; ZebTEC, Tecniplast System, Italy). Fish were fed twice a day with special dry food (Special Diets Services) and supplemented with live brine shrimp. For *in vivo* treatments male breeders were randomly selected and grouped in fours in independent glass tanks (1.5 L). All animals were manipulated in accordance with the Guidelines of the European Union Council (2010/63/EU) following Spanish regulations (RD1205/2005, abrogated by RD 53/2013) and their use was specifically approved by the Research Ethics Committee of the University of León (project number ULE009-2016).

2.3. In vivo exposure of zebrafish to BPA

During treatments, males were kept in 1.5 L tanks (4 males per tank) for 21 days with: i) ethanol 0.014% (v/v) (vehicle used as solvent) (control animals); ii) 100 μg/L BPA (0.44 μM) or iii) 2000 μg/L BPA (8.76 μM). Exposure rounds were developed 15 times to obtain the number of samples required to perform each one of the subsequent analysis. The solutions were totally renewed every day and the stability of BPA during the exposure time was analysed by HPLC (Supplementary Material, SM). On the 22nd day, males were either mated with female breeders for fecundity evaluation or squeezed to collect the sperm following the indications of Carmichael and colleagues (Carmichael et al., 2009). Subsequently, fish were euthanized with ethyl 3-aminobenzoate methanesulfonate (15.31 mM). Gonads were removed, rinsed in PBS (8.37 mM Na_2HPO_4, 1.83 mM KH_2PO_4, 149.9 mM NaCl, pH 7.4; 335 mOsm/kg), then immersed in 0.5% (v/v) of commercial bleach in PBS for 2 min and finally washed again in PBS for 2 min. Testes were used immediately for subsequent analysis after removal of fat and blood. Sperm collected on PBS was immediately centrifuged and cell pellets were stored at −80 °C until RNA extraction (SM Fig. 2).

2.4. In vitro exposure of zebrafish testes to BPA

Adult zebrafish males were dissected to obtain testes for tissue culture experiments, following the indication of Leal MC and colleagues (Leal et al., 2009). Basal tissue culture medium contained 10 mM HEPES, 0.5% bovine serum albumin fraction V (BSA) (Fisher Scientific), 10 nM retinoic acid, 0.4 mg/L amphotericine B (Fungizone®, Invitrogen), 200 U/mL penicillin (Invitrogen) and 200 μg/mL streptomycin (Invitrogen) in Leibovitz-15 (Gibco) medium, pH 7.4. For each male, one testis was incubated under control conditions (basal medium supplemented with 0.014% (v/v) ethanol, vehicle used as solvent) and the contralateral one under experimental conditions: i) basal medium supplemented with 32 μM BPA, or ii) preincubation

with 10 nM G36 for 6 h prior to BPA exposure in the absence of the antagonist (n = 6). The incubation time was extended for 4 days at 26 °C and after that time testes were used for the following analysis.

2.5. Fecundity evaluation

Four males per treatment were mated with non-treated females, each male being kept in a mating tank with two females. The obtained fertilized embryos were counted, rinsed for 2 min with 0.5% (v/v) bleach and 10 s with 70% (v/v) ethanol. Then they were transferred to embryo medium (7.5 mM NaCl, 0.25 mM KCl, 0.5 mM $MgSO_4$, 0.7 mM, KH_2PO_4, 0.02 mM Na_2HPO_4, 0.5 mM $CaCl_2$, 0.35 mM $NaHCO_3$ and 0.05% (v/v) methylene blue; pH 7.2). Embryos were kept at 28 °C in darkness, and the percentage of surviving embryos was obtained at hatching (48 h postfertilization, hpf) (mean ± SEM, n = 4).

2.6. Testis evaluation

2.6.1. Histological testes evaluation

Experimental and control testes were processed for haematoxylin-eosin staining (Pariante et al., 2016) in order to perform morphometric analyses. Testes processing and image analysis are described in SM, data expressed as the percentage of area occupied by a specific cell type relative to the total number of points of the analysed grid (mean ± SEM, n = 3).

2.6.2. Apoptosis assay

Apoptotic cell death was evaluated in testicular cells and in testes sections using the *In Situ Cell Death Detection Kit* (Fluorescein, Roche) (TUNEL assay), following the manufacturer's instruction with some modifications. Testes of individual males (n = 4) were dissociated in Leibovitz's medium (L-15, Gibco) containing 0.2% (w/v) collagenase (Gibco), 10 mM HEPES, 0.5% (w/v) BSA (Fisher Scientific) and DNaseI 1U/mL (Thermo Fisher Scientific). Tissues were incubated at 28 °C for 2 h, with pipette mixing every 15 min. Cell suspensions were filtered through a 0.75 µm filter and washed twice with PBS. Next, pellets were fixed in 4% (w/v) paraformaldehyde and used subsequently for the TUNEL assay as detailed in SM. Data from testicular cells apoptosis are expressed as the percentage of TUNEL-positive cells (mean ± SEM, n = 4).

The previously described paraffin-embedded testis sections were used for evaluating the percentage of apoptotic cells, following the indications stated in SM. Data from tissue section apoptosis are expressed as the ratio of TUNEL-positive cells respect to the total image area (mean ± SEM, n = 3).

2.6.3. RNA/protein extraction and cDNA synthesis

Total RNA and protein from testes of *in vivo* experiment (n = 4) or for *in vitro* exposure (n = 4) were extracted using TRIzol™ reagent (Invitrogen), following the manufacturer's instructions. Total RNA was solubilized in 30 µL DEPC (diethyl pyrocarbonate) – water and total protein was resuspended in 50 µL protein buffer (8 M urea, 4% sodium dodecyl sulphate (SDS) and protease/phosphatise inhibitor cocktail 1X (Thermo Scientific)). RNA integrity was confirmed by agarose gel electrophoresis before reverse transcription to complementary DNA (cDNA) (data not shown). Total RNA quantity was determined spectrophotometrically at 260 nm (Nanodrop 1000, Thermo Scientific) and total protein concentration was assessed by Pierce™ BCA Protein Assay Kit (Thermo Scientific), absorbance being measured at 562 nm on a Synergy™ HT microplate reader (BioTek).

Approximately 1 µg of total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) following the recommended protocol and stored at −20 °C until further analysis. Conditions of reverse transcription were set as: 25 °C for 10 min, 37 °C for 120 min and 85 °C for 10 min.

2.6.4. Gene expression evaluation

Quantitative real-time PCR (RT-qPCR) was performed to analyse gene expression of different transcripts related to: i) estrogen or estrogen-related receptors (*esr1*, *esr2a*, *esr2b*, *esrrga*, *gper-1*); ii) cell cycle/spermatogenesis (*ccnb1*, *ccnd1*; *scyp3*); iii) apoptosis (*noxa*, *bcl2*); iv) DNA methylation remodelling (*dnmt1*, *dnmt3*, *dnmt5*, *dnmt8*); v) histone acetylation/deacetylation (*kat2b*, *hdac6*, *kat6a*, *hdac4*) and vi) histone methylation/demethylation (*ezh2*, *kdm6b*, *kmt2a*, *kdm5a*). RT-qPRC was performed in a StepOnePlus™ System (Applied Biosystems). Run conditions and primer sequences are annotated in SM. The normalized mRNA levels were compared using $2^{-\Delta Ct}$ ($\Delta C_t = C_t$ target $- C_t$ reference) and data were expressed as mean ± SEM (n = 4).

2.6.5. Western blot

Thirty µg of protein were loaded onto a 12% polyacrylamide gel (SDS-PAGE, Bio-Rad) and electrophoresis was set at 100 V for 180 min. Proteins were transferred to a nitrocellulose membrane (Invitrogen) using a wet transfer system (Bio-Rad) at 30 V for 16 h. Ponceau S staining (0.1% (w/v) Ponceau S in 5% (v/v) acetic acid) was used for checking protein transfer. Membranes were blocked in 5% (w/v) BSA in 0.2% (v/v) Tween-20 in Tris-buffered saline (TBS-T) for at least 1 h at room temperature. After that, they were incubated overnight at 4 °C with the corresponding primary antibody diluted at 1/1000 (rabbit polyclonal to G-protein coupled receptor 30, ab137479; rabbit polyclonal to ERK1 + ERK2, ab17942; mouse monoclonal to ERK1 (pT202/pY204) + ERK2 (pT185/pY187), ab50011; rabbit monoclonal to CREB, ab32515; rabbit monoclonal to CREB (phosphor S133), ab32096). Anti-rabbit or anti-mouse secondary antibody conjugated with horseradish peroxidase (Invitrogen) at a dilution of 1/3000 was incubated for 1 h at room temperature and was developed using the SuperSignal™ West Pico PLUS Chemiluminescent substrate (Thermo Scientific). Vinculin (rabbit monoclonal to vinculin, ab129002) or tubulin (rabbit polyclonal to beta tubulin, ab6046) levels were used as loading control. Exposed and developed films were scanned in a GS800 densitometer (Bio-Rad). Band intensity was quantified by Image J software (Gallo-Oller et al., 2018). Data were obtained as OD and expressed as mean ± SEM (n = 4).

2.6.6. Global histone H3 acetylation

Testes were dissociated as stated above (see 2.6.b). Once the cell pellet was obtained, histones were extracted according to the protocol described by Schechter and colleagues (Shechter et al., 2007). Protein concentration was measured with Pierce™ Coomassie (Bradford) Protein Assay Kit (Thermo Scientific) at 595 nm in a microplate reader Synergy™ HT (BioTek) and diluted to obtain a final concentration between 200 and 400 ng/µL. Global histone H3 acetylation was determined using the Histone H3 Acetylation Assay Kit (ab115102, Abcam) following the manufacturer's instructions. For quantification, acetylated histone H3 standards were used from 100 ng to 10 ng and a calibration curve was determined. Acetylation was measured at 450 nm using a microplate reader Synergy™ HT (BioTek). Data are expressed as acetylated H3 (ng)/total protein (mg) (mean ± SEM; n = 5).

2.6.7. Histone acetyltransferase (HAT) activity assay

Testicular nuclear extracts were obtained following a routine protocol. Briefly, testes were dissociated as previously stated and the obtained cell pellets were resuspended in 50 µL lysis buffer A (10 mM HEPES pH 7.5, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% (v/v) NP-40 and 1X protease inhibitor (Thermo Scientific)). After an incubation period of 20 min on ice, the suspensions were centrifuged at 12000*g* for 10 min at 4 °C. The supernatants, corresponding to the cytoplasmic extracts, were kept at −80 °C for further analysis and the nuclei pellets were resuspended in 25 µL lysis buffer B (20 mM HEPES pH 7.5, 400 mM NaCl, 1 mM EDTA, 1 mM DTT and 1X protease inhibitor (Thermo Scientific)). The suspensions were incubated for 30 min on ice, with vortex mixing (5 s) every 5 min. After that period, extracts were sonicated 3 × 10 s and centrifuged at 12000*g* for 15 min at 4 °C. Supernatants, corresponding to nuclear extracts, were maintained at −80 °C until their analysis. Protein quantity in cytoplasmic and nuclear extracts was quantified using the Bradford method as indicated before. Histone acetyltransferase activity was evaluated with the EpiQuik™ HAT Activity/Inhibition Assay Kit (EpiGentek), following the manufacturer's instructions. HAT standards (5–0.5 ng) were used for curve calibration and 2 µL of nuclear extracts were assayed. Enzymatic activity was determined at 450 nm using a microplate reader Synergy™ HT (BioTek). Absorbance values were used for HAT quantification (ng) and these values were normalized to the initial protein quantity (mg). Data are expressed as HAT (ng)/total protein (mg) (HAT activity; mean ± SEM, n = 3).

2.6.8. Evaluation of specific epigenetic marks

Testes were dissociated as stated above (see 2.6.b) and used for the quantification of 5 mC, H3K9ac, H3K14ac, H3K27ac, H3K27me3 and H4K12ac by immunolabelling, following the indications described in SM. Data obtained from flow cytometry quantification are expressed as the ratio of the corresponding channel intensity respect to the size of the gated population in arbitrary units (APC/FSC or AlexaFluor®488/FSC, for 5 mC or acetylated histone evaluation, respectively) (mean ± SEM, n = 4 and n = 6 in samples from *in vivo* or *in vitro* experiments, respectively).

2.7. Sperm assessment

2.7.1. RNA extraction and cDNA synthesis

Sperm from four males was pooled and total RNA from approximately 10^6 sperm cells was extracted with a RNeasy Mini Kit (Qiagen), following the manufacturer's indications. RNA was solubilized in 20 µL DEPC-water, RNA integrity was confirmed by agarose gel electrophoresis and RNA quantity was determined at 260 nm (Nanodrop 1000, Thermo Scientific). Approximately, 1 µg of RNA was reverse transcribed as described for testicle procedure.

2.7.2. mRNA level evaluation

Quantitative real-time PCR (RT-qPCR) was performed for analysing gene expression of those genes transcriptionally altered after BPA treatment in testes: i) DNA methylation remodelling (*dnmt5*); ii) histone acetylation (*kat6a*); iii) histone demethylation (*kdm6b*); iv) estrogen-related receptors (*esrrga*, *gper-1*) and v) spermatogenesis (*scyp3*). RT-qPRC was performed in a StepOnePlus™ System (Applied Biosystems) using oligonucleotides described in SMTable I. The reaction mixture was the same as that used previously, unless the quantity of cDNA was 600 ng. PCR conditions were as previously stated (see SM). Three technical replicates were performed for each sample and *18S* rRNA was used as endogenous reference gene. The normalized mRNA levels were compared using $2^{-\Delta Ct}$ ($\Delta C_t = C_t$ target – C_t reference) and data are expressed as mean ± SEM (n = 3).

2.7.3. DNA fragmentation

DNA fragmentation was analysed using the Comet assay (or SCGE, *Single Cell Gel Electrophoresis*), following the indications of Fernández-Díez and coworkers (Fernández-Díez et al., 2018). Sperm was obtained and subsequently diluted to 10^6 cells/mL in PBS. Technical duplicates and positive controls (20 µM H_2O_2, 15 min) were included in the assay. Once slices were fixed, 20 µL 0.5 µg/mL DAPI were used for nuclear staining and comet visualization. Approximately, images of 50 cells per slide were acquired with a fluorescence microscope (Eclipse E800, Nikon; equipped with a digital camera DXM1200F, Nikon), fitted with a 510–560 nm excitation filter and a 590 nm barrier filter. To quantify DNA damage, the percentage of tail DNA (% DNAt) was measured using the free CaspLab software (1.2.3beta2; http://www.casp.of.pl) (mean ± SEM, n = 4).

2.8. Statistical analysis

Statistical evaluation was carried out with SPSS version 24.0 (IBM, EEUU). Normality of data was tested by the Shapiro-Wilk test. For non-parametric data, a Kruskal-Wallis test was performed using Dunn's post hoc test ($p < 0.05$). For parametric data, an analysis of variance (ANOVA) with a Bonferroni or DMS post hoc test was run ($p < 0.05$). Results are shown as media ±SEM.

3. Results

3.1. BPA stability

BPA stability was confirmed by HPLC assay during a 24 h cycle of exposure. Monitoring curve did not reveal any alteration of BPA concentration in water (SM Figs. 1 and 2), averages values being 83.76 ± 1.25 and 1684.81 ± 13.02 for 100 µg/L and 2000 µg/L experimental conditions, respectively.

3.2. BPA affects fecundity and embryo development

Males treated with 100 µg/L BPA did not show reduced fecundity but survival at hatch was reduced from 47.03% ± 8.7 (mean ± SEM) in batches from control males to 6.97% ± 2.6 in batches from treated males ($p = 0.004$, gl = 11) (Fig. 1A and B). Treatment with the highest BPA dose severely affected fertilization capacity (2000 µg/L BPA reduced in 81.6% the number of fertilized embryos respect to control), and none of the obtained embryos survived at hatching ($p = 0.001$, gl = 11) (Fig. 1A and B).

3.3. BPA compromises spermatogenetic activity

After paternal BPA exposure, morphometric study revealed that neither spermatogonia, spermatids nor spermatozoa percentages were altered after *in vivo* exposure to BPA; however, the highest dose of the toxic significantly reduced the proportion of spermatocytes from a ratio of 0.22 ± 0.02 (mean ± SEM) in vehicle to 0.13 ± 0.01 in 2000 µg/L of BPA ($p = 0.018$, gl = 8) (Fig. 1C and D). Moreover, TUNEL assay carried out in dissociated-testicular cells revealed an increase in apoptotic cells after treatment with the highest dose of BPA that affected the three evaluated populations ($p = 0.024$, $p = 0.033$ and $p = 0.047$ for haploid, diploid and tetraploid, respectively, gl = 8) (Fig.

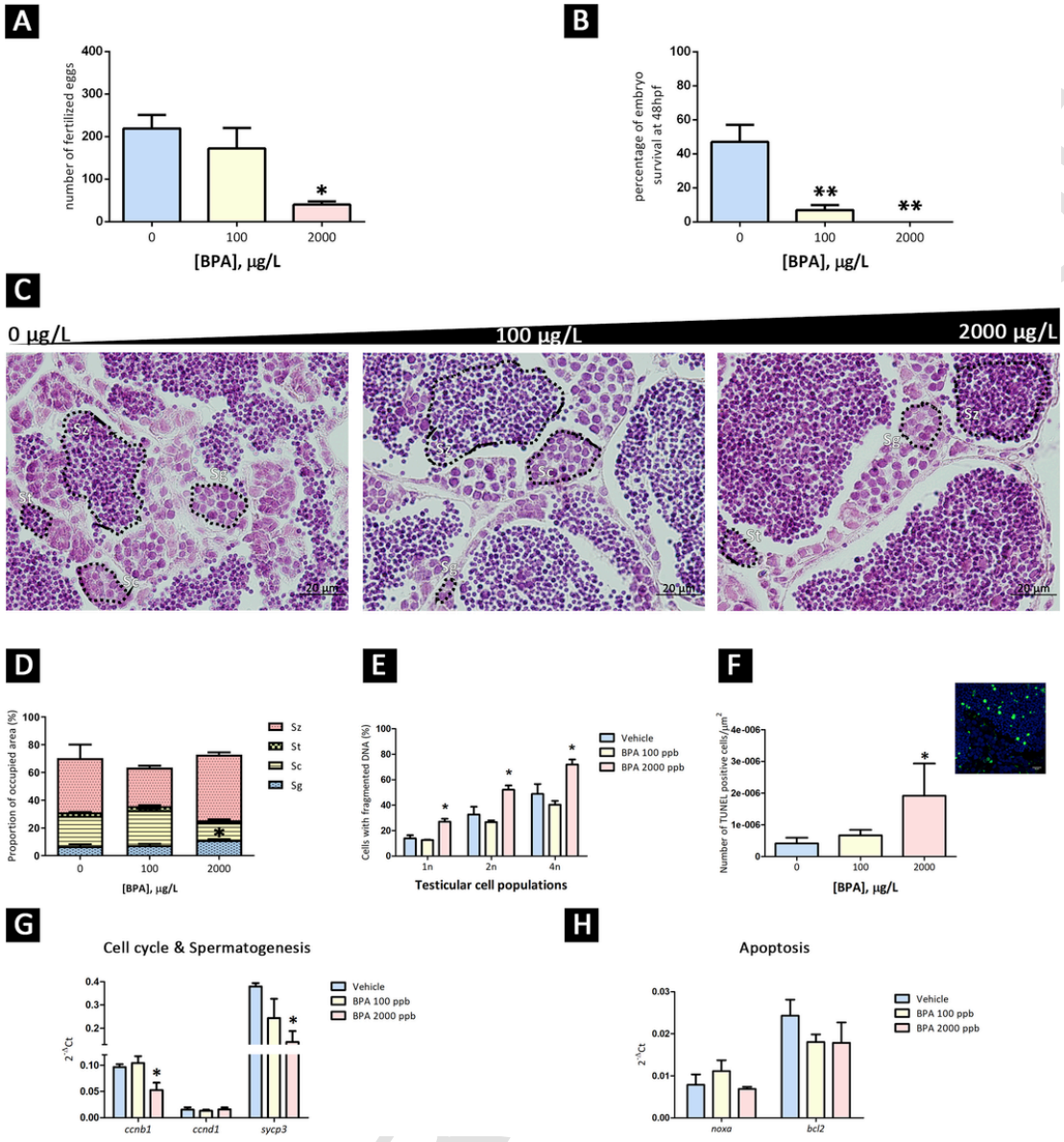

Fig. 1. Progeny evaluation and morphometric analysis in testes after *in vivo* exposure to BPA of zebrafish males. (**A**) Number of fertilized eggs and (**B**) percentage (%) of embryo survival at hatching is represented after *in vivo* control or experimental conditions. (**C**) Representative histological images of sections of control and treated zebrafish testicles, stained with haematoxylin and eosin. Sg: spermatogonias; Sc: spermatocytes; St: spermatids; Sz: spermatozoa. Scale bar: 20 µm. (**D**) Quantification of testicular cell types after BPA exposure. Proportion (%) of area occupied by cells is represented for each experimental condition (n = 3). (**E**) Apoptosis evaluation by TUNEL and flow cytometry in dissociated testicles, expressed as the percentage of cells with fragmented DNA for all testicular cell populations (n, haploid; 2n, diploid; 4n, tetraploid) after BPA treatment (n = 4, acquiring at least 10000 events in triplicate). (**F**) Apoptosis evaluated in testicle slices, expressed as the number of cells with fragmented DNA respect to the microscope field size (μm^2) (n = 3). An example of a fluorescence image corresponding to a 2000 µg/L BPA-exposed testicle is shown. (**G**) Relative mRNA level of cell cycle and spermatogenesis genes (*ccnb1, ccnd1, sycp3*) and (**H**) apoptosis genes (*noxa*, *bcl2*) were calculated using $2^{-\Delta Ct}$ method relative to *actb2* gene (n = 4). Data are expressed as mean ± SEM. Asterisks show significant differences with respect to control samples (*$p < 0.05$; **$p < 0.01$).

1E). We also confirmed this observation when the number of apoptotic cells per field was analysed in testicle slices, the highest dose of BPA promoting a significant increase in apoptosis ($p = 0.014$, gl = 8) (Fig. 1F). Moreover, a downregulation of transcript related to cell cycle/spermatogenesis was confirmed after paternal exposure to 2000 µg/L of BPA (*ccnb1* (cyclin B1) ($p = 0.045$, gl = 10) and *sycp3* (synaptonemal complex protein 3) ($p = 0.041$, gl = 11)) (Fig. 1G); however, neither *ccnd1* (cyclin D1), *noxa* (*pmaip1*, phorbol-12-myristate-13-acetate-induced protein 1) nor *blc2* (apoptosis regulator a) showed any change in expression (Fig. 1H).

3.4. Effect of BPA on estrogen and estrogen-related receptors

Expression of estrogen and estrogen-related receptors as well as of genes downstream of their pathways was affected by BPA treatment. No changes in the expression profile either of *esr1* (estrogen receptor 1), *esr2a* (estrogen receptor 2a) or *esr2b* (estrogen receptor 2b) were observed. In contrast, an upregulation in *esrrga* (estrogen-related receptor gamma a) and *gper1* (G protein-coupled estrogen receptor 1) was observed after treatment with the highest dose of BPA ($p = 0.032$ and $p = 0.014$, respectively, gl = 11) (Fig. 2A).

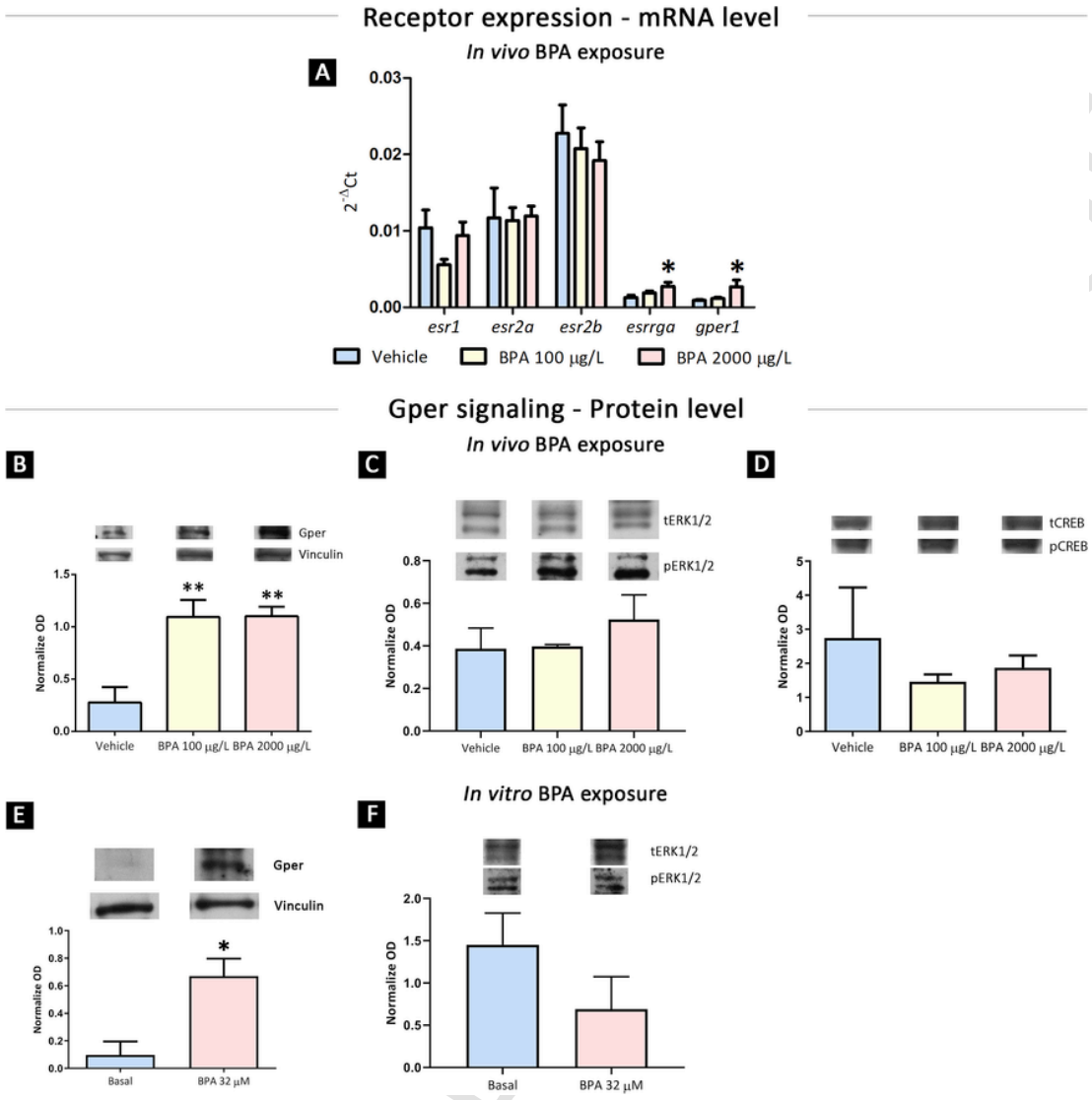

Fig. 2. Relative expression of receptor genes and protein level of Gper signalling after *in vivo* and *in vitro* BPA exposure. (**A**) Relative mRNA level of estrogen or estrogen-related receptors (*esr1*, *esr2a*, *esr2b*, *esrrga*, *gper1*) were calculated using $2^{-\Delta Ct}$ method relative to *actb2* gene (n=4). Effect of *in vivo* (**B-D**) or *in vitro* (**E-F**) BPA exposure on protein levels are represented. Bands and their corresponding optical densities normalized to vinculin (124 kDa) are represented. Gper (42 kDa) bands and their corresponding normalized ratios are shown (n=4) after *in vivo* (**B**) or *in vitro* (**E**) exposure. ERK1/2 (42–44 kDa) and pERK1/2 (44 kDa) bands and their corresponding ratios pERK1/2:tERK1/2 were represented after *in vivo* (**C**) or *in vitro* (**F**) BPA exposure. CREB (37 kDa) and pCREB (37 kDa) and pCREB:tCREB ratio are illustrated after *in vivo* BPA exposure (**D**). Results are expressed as mean ± SEM and asterisks show significant differences when compared to the control group (*$p < 0.05$; **$p < 0.01$).

3.5. BPA interference on GPER signalling

Western blot analysis revealed an increase of Gper protein level promoted by BPA both *in vivo* ($p=0.007$ and $p=0.007$, for 100 µg/L and 2000 µg/L BPA, respectively, gl=11) (Fig. 2B) and *in vitro* ($p=0.023$, gl=7) (Fig. 2E). pERK1/2:tERK1/2 and pCREB:tCREB assessment did not show a downstream activation of the ERK pathway after paternal exposure to BPA (Fig. 2C and D). Similar results were observed when testes were *in vitro* exposed to 32 µM BPA (Fig. 2F).

3.6. Effect of BPA on epigenetic remodelling enzymes

BPA exposure significantly modifies testicular expression of some genes involved in epigenetic remodelling. With regard to methylation, *dnmt5* (DNA (cytosine-5)-methyltransferase beta, duplicate b.3) and *kdm6b* (lysine (K)-specific demethylase 6B) were upregulated after treatment with the highest dose of BPA ($p=0.029$, gl=8, and $p=0.018$, gl=11, respectively) (Fig. 3A and C). Regarding enzymes related to acetylation, *kat6a* (K(lysine) acetyltransferase 6A) underwent a downregulation after exposure to the highest dose of BPA ($p=0.046$, gl=10), whereas *hdac4* (histone deacetylase 4) transcript showed an increase with the same treatment ($p=0.027$, gl=8) (Fig. 3B).

3.7. Global hyperacetylation after BPA treatment

The study of global H3 acetylation confirmed that the two BPA doses promoted H3 hyperacetylation *in vivo* ($p=0.044$ and $p=0.046$ for 100 µg/L and 2000 µg/L of BPA, respectively, gl=14) (Fig. 4A). Moreover, analysis of global HAT activity in nuclear extracts from

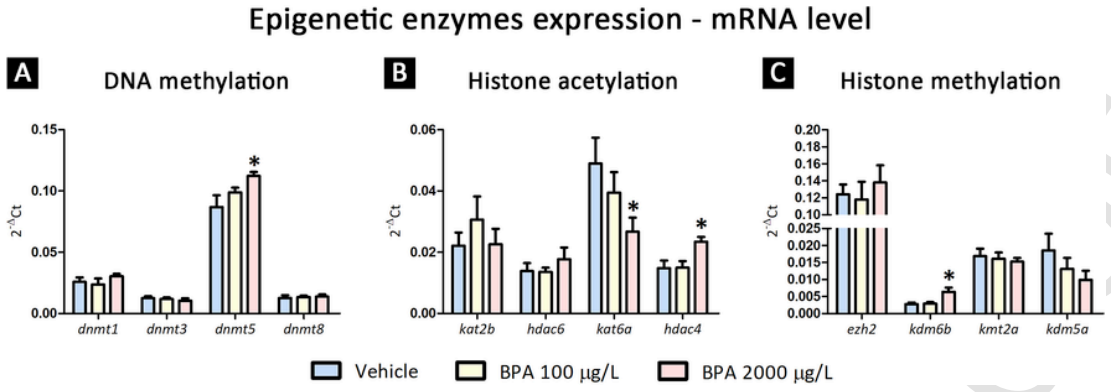

Fig. 3. Relative expression of zebrafish genes after male BPA exposure in testes. Relative mRNA level of (**A**) DNA methyltransferases genes (*dnmt1*, *dnmt3*, *dnmt5*, *dnmt8*), (**B**) histone acetylation remodelling enzymes (*kat2b*, *hdac6*, *kat6a*, *hdac4*) and (**C**) histone methylation remodelling enzymes (*ezh2*, *kdm6b*, *kmt2a*, *kdm5a*) were calculated using $2^{-\Delta Ct}$ method relative to *actb2* gene. Results are expressed as mean ± SEM and asterisks show significant differences when compared to the control group (n = 4, $p < 0.05$).

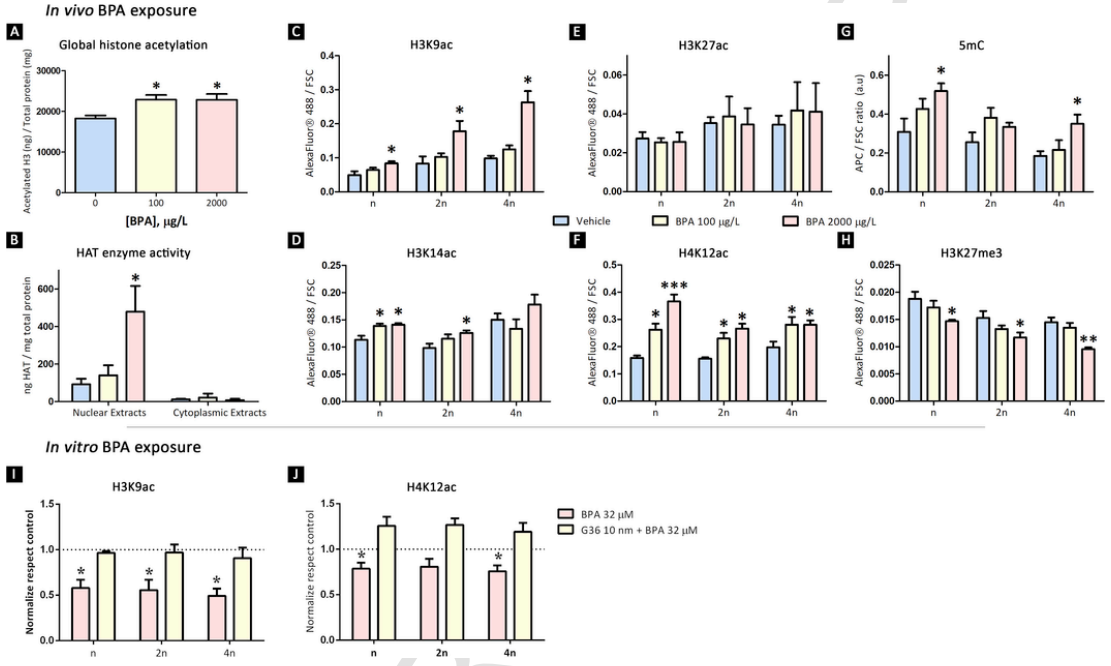

Fig. 4. Evaluation of epigenetic modifications in zebrafish testes after BPA treatment of zebrafish males. (A) Global acetylation of histone H3. Data are expressed as the quantity of acetylated histone H3 (ng) respect to the total amount of histones used in the assay (mg) (n = 5). (**B**) Histone acetyltransferase (HAT) activity was determined in testes nuclear or cytoplasmic extracts. Data are expressed as the quantity of HAT enzyme (ng) respect to the total amount of protein (mg) (n = 3). Specific epigenetic marks were evaluated by flow cytometry after (**C-H**) *in vivo* or (**I-J**) *in vitro* BPA exposure. Graphs represent the ratio of fluorescence intensity mean respect to the mean of the gated population size (FSC) for the three testicular cell populations studied (n: haploid, 2n: diploid and 4n: tetraploid cells) and after vehicle or BPA treatment (n = 4 for *in vivo* experiments or n = 6 for *in vitro* experiments). Different fluorophores were used, AlexaFluor® 488 being used for (**C, I**) H3K9ac, (**D**) H3K14ac, (**E**) H3K27ac, (**F, J**) H4K12ac and (**H**) H3K27me3 and APC for (**G**) 5 mC determination. Bars represent the mean ± SEM and asterisks show significant differences compared to the untreated samples (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

zebrafish testes after male exposure allowed us to verify the increase in HAT activity after 2000 µg/L of BPA treatment ($p = 0.014$, gl = 8), in accordance with the detected hyperacetylation on histones. As expected, there was scarce HAT activity on cytoplasmic extracts (Fig. 4B).

3.8. Alteration of specific epigenetic marks promoted by BPA

BPA exposure significantly modified the epigenetic status of specific DNA and histone marks of zebrafish testes. Regarding histone acetylation, an overall increase was observed in the studied marks. H3K9ac increased in the three analysed testicular cells ($p = 0.024$ for haploid; $p = 0.016$ for diploid and $p = 0.014$ for tetraploid cells, gl = 10) with the highest dose of BPA (Fig. 4C). H3K14ac was intensified in haploid cells with the two tested doses and in diploid cells with the highest dose of BPA ($p = 0.030$, $p = 0.029$ for haploid cells after exposure to 100 µg/L and 2000 µg/L of BPA, respectively and $p = 0.037$ for diploid cells, gl = 10) (Fig. 4D). H3K27ac did not reveal any change of pattern in any of the cells respect to the vehicle (Fig. 4E). Moreover, hyperacetylation of lysine 12 on histone H4 (H4K12Ac) was revealed in all testicular cells after exposure to the two BPA doses ($p = 0.016$ and $p = 0.000134$ for haploid cells; $p = 0.032$ and $p = 0.003$ for diploid cells and $p = 0.028$ and $p = 0.028$ for tetraploid cells after 100 µg/L or 2000 µg/L of BPA, respectively, gl = 11) (Fig. 4F). DNA

methylation, evaluated by analysing the 5-methylcytosine mark (5 mC), showed hypermethylation in both haploid and tetraploid cells after 2000 µg/L BPA exposure ($p=0.025$ and $p=0.017$, respectively, gl=10) (Fig. 4G). It was also observed that 2000 µg/L of BPA caused a decrease of H3K27me3 in all testicular cells respect to the control testicles ($p=0.022$; $p=0.028$ and $p=0.003$, respectively, gl=11) (Fig. 4H).

In vitro exposure of testes to 32 µM BPA promoted a decrease of H3K9ac in all testicular cells ($p=0.005$, $p=0.026$ and $p=0.027$ for haploid, diploid and tetraploid cells, respectively, gl=10) which was reverted when testes were previously treated with G36 (Fig. 4I). Likewise, BPA caused a reduction of H4K12ac in haploid and tetraploid cells ($p=0.042$ and $p=0.026$, respectively, gl=10), which was restored by the GPER-antagonist (Fig. 4J). In order to confirm whether the altered transcription in testicle implied the presence of a modified pattern of transcripts in spermatozoa, expression of those genes that presented an up/downregulation in testes was evaluated. However, no differences were observed except for *sycp3* which showed a significant downregulation after 100 µg/L and 2000 µg/L of BPA ($p=0.014$ and $p=0.014$, respectively, gl=8) (Fig. 5A).

The comet assay revealed that treatment with the highest dose of BPA also promoted an important DNA fragmentation during spermatogenesis, increasing from 8.64%±2.36 in sperm of control males to 50.00%±3.24 in sperm from those treated with 2000 µg/L ($p=0.010$, gl=11) (Fig. 5B and C).

4. Discussion

BPA is an endocrine disruptor, widely spread through the environment, with proven effects on reproductive functions. Testicular atrophy and small gonadal size have been attributed to direct BPA exposure which reduced testosterone levels in rat and mice (Manfo et al., 2014; Peretz et al., 2014). These effects on male reproduction have been related to its capacity to bind to estrogen receptors (ERs), modifying the physiological control exercised by the hypothalamic-pituitary-gonadal axis and, consequently, impairing spermatogenesis (Rahman et al., 2017). The tested doses are very high compared to those nowadays allowed for human consumption but are in range of concentrations occasionally reported in contaminated water bodies, relevant for aquatic organisms. As summarized by Willhite and colleagues (Willhite et al., 2008) typical surface water concentrations range from 0.0005 to 0.4 µg/L but from 0.0005 to 98 µg/L (the lower tested dose) was detected in river and surface water, wastewater and tap water. 100 µg/L dose has been considered safe in drinking water for human consumption relatively recently (Willhite et al., 2008), and 2000 µg/L is close to that reported in water leachates generated by municipal solid waste plants (Fudala-Ksiazek et al., 2017; Morin et al., 2015). Our results suggest that environmental exposure of male zebrafish to the highest dose severely impaired spermatogenesis, affecting the production of spermatozoa and modifying the information contained in sperm cell. As a result, fertilization ability was dramatically reduced and the obtained embryos were unable to survive at hatching. Effects promoted by the lower dose seem to be less critical: the treatment caused epigenetic changes in the absence of other detectable modifications, males maintained their fertilization capacity but embryo development was impaired.

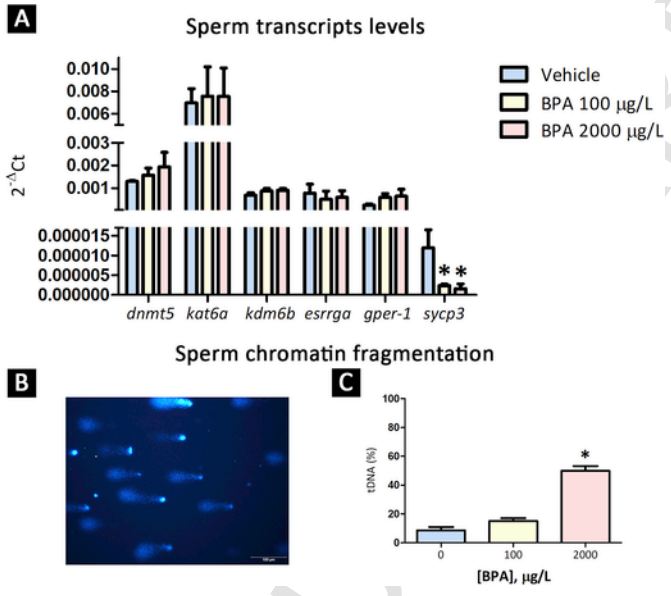

Fig. 5. Evaluation of remnant mRNAs and chromatin integrity in zebrafish sperm after BPA exposure. (A) mRNA level in spermatozoa where obtained relative to *18S rRNA* using $2^{-\Delta Ct}$ method (n=3). (**B-C**) Sperm chromatin fragmentation evaluated by Comet assay. (**B**) Representative fluorescence image of spermatozoa. Scale bar: 100 µm. (**C**) Percentage of tail DNA evaluated in sperm cells from males exposed to control conditions or BPA (n=4). Bar results are expressed as mean±SEM and asterisks show significant differences regarding vehicle samples ($p<0.05$).

Testes from males exposed to 100 µg/L showed a normal spermatogenic pattern, whereas an intense alteration of spermatogenic cell proliferation was observed in males exposed to 2000 µg/L BPA: spermatocyte population was reduced and apoptosis was clearly enhanced, suggesting a disruption of the meiotic process. Several reports have previously described the induction of spermatocytes apoptosis together with the disruption of meiotic progression after BPA treatment in mammals (mice and rats) (Ali et al., 2014; Liu et al., 2013; C. Wang et al., 2017a,b; Xie et al., 2016) and fish (Zhang et al., 2016). Moreover, *in vitro* BPA exposure promoted meiotic arrest in rat seminiferous tubules (Ali et al., 2014) or in zebrafish oocytes (Thomas, 2017). All these effects could reflect an alteration of the mitotic and meiotic progression of spermatogenesis, which could be related to the observed downregulation of two specific transcripts: *ccnb1*, cyclin involved in G2-M cell cycle transition, and *sycp3*, coding a protein of the synaptonemal complex, generated at prophase I. BPA interference with apoptotic pathways has been also documented. Fas/FasL and caspase-3 expression have been upregulated increasing apoptosis in mice testes (Li et al., 2009) and germ cells (Wang et al., 2010) after BPA exposure. An increase in caspase activity has been also observed after *in vitro* BPA exposure of mouse and rat spermatocytes (Qian et al., 2015; Wang et al., 2014) as well as of mouse spermatogonial stem cells (Gong et al., 2017) and of human spermatozoa (Barbonetti et al., 2016). Our study revealed that the increase in apoptosis after incubation in 2000 µg/L of BPA affected all types of testicular cells. In another cyprinid (*Gobiocypris rarus*), exposure to 225 µg/L BPA induced spermatocytes apoptosis and altered expression of several genes, which suggests upregulation of the intrinsic mitochondrial apoptotic pathway (*bcl2*, *bax*, *casp9*, *cytc* and *mcl1b*) (Zhang et al., 2016). In our case, however, we did not observe any alteration in gene expression of proapoptotic (*noxa*) or antiapoptotic (*bcl2*) genes, questioning the activation of the intrinsic pathway.

Binding to hormone receptors is considered the primary cause of BPA toxic effects (Manfo et al., 2014). Evidence has suggested that BPA can bind to a wide range of estrogen receptors, from the classical nuclear receptors ERα and ERβ, to non-classical membrane ERs, as well as to the membrane G-protein–coupled estrogen receptor GPER (as reviewed by MacKay and Abizaid (2018)). Zebrafish exposure to natural and synthetic estrogens showed the overexpression of *esr1* in

organs such as liver, intestine, brain and testis (Chandrasekar et al., 2010). However, our analysis concerning mRNA levels in testicle did not reveal any change in classical ER expression. BPA binding affinity for ERs is 1000–10000-fold lower compared to the natural ligand 17β-estradiol. Nevertheless, an upregulation of *errga* and *gper1* was clearly noticed after 2000 μg/L BPA exposure. The increased *gper1* gene expression was also confirmed at the protein level by western blot for both *in vivo* and *in vitro* approaches. BPA has been described as binding to GPER with an affinity 8–50 times higher than to ERs (Thomas and Dong, 2006). Regarding reproduction and, specifically, spermatogenesis, the specific role of GPER on this process is not clear. GPER expression has been identified in spermatogonia, spermatids, Sertoli cells, Leydig cells (Chimento et al., 2014; 2011; Prossnitz and Arterburn, 2015). Moreover, GPER is able to regulate proliferation and apoptosis during rat spermatogenesis (Chimento et al., 2010), modifying the expression of cyclins and apoptotic factors through the EGFR/ERK pathway (Chimento et al., 2011; Wang et al., 2017a,b). All this evidence suggests that BPA could act as a ligand to GPER modulating cell proliferation and apoptosis via MAPK/ERK pathway. Nevertheless, our results, both *in vivo* and *in vitro,* rule out activation of ERK as mediating these effects: increased expression of GPER and the absence of ERK phosphorylation suggest the activation of different downstream pathways. GPER trans-activation of EGFR promotes subsequent phosphorylation of MAPK/ERK targets as well as activation of PI3K/AKT signalization (Chimento et al., 2010; Feldman and Limbird, 2017; Prossnitz et al., 2008; Prossnitz and Barton, 2011; Thomas, 2017). It has also been described the stimulation of adenyl cyclase and mobilization of intracellular calcium by GPER (Chimento et al., 2014; Feldman and Limbird, 2017). These modes of GPER signalization are implicated in mediating rapid non-genomic responses or in genomic responses regulating gene expression and should be further explored. Moreover, the anti-androgenic effects of BPA (Fang et al., 2017; Sun et al., 2006) could also mediate spermatogenesis impairment and should be seriously considered.

Spermatogenesis entails huge epigenetic remodelling (in the form of DNA/histone methylation or histone acetylation) which is essential to allow the required transcriptional and conformational changes (Hazzouri et al., 2000; Schagdarsurengin and Steger, 2016). Therefore, the process represents a susceptible window of exposure to epigenotoxicants. In the present study we provide detailed information of epigenetic marks in testicular cells after adult male exposure. The window of exposure used in this study is sufficiently long to cover the entire zebrafish spermatogenesis, estimated at 21 days (Schulz et al., 2010). After treatment, we observed, for the first time, a global hyperacetylation of histones in testes. This increase in histone acetylation was in accordance with the increment in testicular histone acetylatransferase enzymatic activity observed after BPA treatment. To better understand this alteration, we evaluated the level of specific histone acetylation marks, a found an increase of H3K9ac, H3K14ac and H4K12ac in testicular cells after *in vivo* BPA exposure, H4K12 and H3K14 acetylation increasing even after treatment with the lower doses. The scarce and fragmentary data available regarding histone acetylation after BPA exposure in mammals reveal differential results: a similar increase of global H3K9ac and H4K12ac was observed after oocyte BPA exposure in mice (Liu et al., 2017), but decreases of H3ac and H3K14ac in *StAR* promoter were reported after BPA mice embryo treatment (Hong et al., 2016) and in H3K9ac and H3K27ac after BPA diet intake in rat testes (Chen et al., 2017). Our *in vitro* model provides inverse results to those reported *in vivo*, with BPA causing a decrease rather than an increase of the analysed marks (H3K9ac and H4K12ac). The use of *in vitro* models requires different exposure conditions (doses and times), that could promote differential effects. These changes were reverted to the basal acetylation level observed in control testes when organs were treated with GPER-antagonist G-36, thus indicating the involvement of GPER signalling in mediating the observed changes in histone acetylation.

Additionally, we observed a decrease of H3K27me3 in haploid, diploid and tetraploid testicular cells, which had not been previously reported after male BPA treatment. Reduction of the trymethylated histone mark and the increase of acetylated histones had been previously described in cancer cell lines after estrogen supplementation (Dagdemir et al., 2013) and suggested a more permissive scenario to transcriptional activity. Regarding to the effects of male BPA treatment on DNA methylation, the available data are some conflicting. Our findings show a hypermethylation (in terms of 5-methylcytosine) of testicular DNA. This observation had been previously reported, in a specific gene promoter, in ovaries and testes of rare minnow *Gobyocypris rarus* (Liu et al., 2014a,b; Yuan et al., 2016; Zhang et al., 2017). However, other studies reveal a decrease in DNA methylation in fish gonads (Laing et al., 2016; Liu et al., 2016; Yuan et al., 2017) or the stability of the pattern of DNA methylation, both in rat/mice (Dere et al., 2018; Zhang et al., 2013) and in zebrafish (Lombó et al., 2015). To sum up, BPA exposure through spermatogenesis affects the epigenetic pattern of germinal cells during differentiation. Any epigenetic alteration during pre-meiotic and meiotic phases could jeopardize haploid cells, modifying the epigenome of the mature gametes, which could have serious consequences not only on the spermatogenesis process itself but also, as reviewed by Champroux and colleagues (Champroux et al., 2018), on the forthcoming progeny.

The transcriptional status of the enzymes involved in histone acetylation show paradoxical changes of expression: downregulation of *kat6a* (histone acetyltransferase) and upregulation of *hdac4* (histone deacetyltransferase), suggestive of a totally different histone acetylation profile than the observed one. This effect could reflect the activation of a compensating mechanism to restore normal histone acetylation status, especially considering that analysis of the enzymatic acetyltransferase activity revealed a clearly enhanced activity in treated males. Nevertheless, *kdm6b* (histone demethylase) was upregulated in zebrafish testes, according to the decrease in H3K27me3, and *dnmt5* levels (de novo DNA methyltransferase) were increased, in agreement with the rise in DNA methylation after BPA treatment. Alteration of DNA methyltransferases transcripts had been previously observed in F2 zebrafish offspring after paternal BPA exposure, where several enzymes were downregulated (Chen et al., 2015).

Altered transcriptional and epigenetic landscape in somatic and pre-meiotic cells, which are transcriptionally active, could modify the number of transcripts transmitted to spermatozoa at the end of spermatogenesis. Our analysis of spermatic transcripts on ejaculates showed a sharp decrease in one of them (*sycp3*) in males exposed to BPA. Sperm RNAs are widely accepted to be part of the paternal epigenetic information delivered to the oocyte and maintained in the zygote (Avendaño et al., 2009; Hamatani, 2012; Johnson et al., 2011; Ostermeier et al., 2004) and, as noticed by Swan and Lai (Swann and Lai, 2016), could be translated by the embryo upon fertilization. Thus, paternal exposure to BPA during spermatogenesis promotes an endocrine disruption which missregulates specific transcripts in testes and sperm, unbalancing the information received by the zygote upon fertilization. Indeed, mRNA level alteration in sperm has been proposed as being responsible for the malformations observed in progeny after paternal exposure to BPA or 17-α-ethinylestradiol (Lombó et al., 2015; Valcarce et al., 2017). Moreover, post-meiotic haploid testicular cells, which comprise late spermatids and spermatozoa, displayed a significantly altered epigenetic pattern after BPA exposure, even at the lowest doses. The observed effects on the transcriptome

and epigenome could thus lie behind the high mortality observed in the progeny of treated males during embryo development.

The effects promoted by 2000 μg/L BPA also include a sharp increase in sperm DNA fragmentation. BPA has been reported to impair DNA integrity during rat spermatogenesis (Liu et al., 2014a,b; Liu et al., 2013). The mechanism could be related, among other factors, to the imbalance of the DNA repairing activities (Murata and Kang, 2018) or to the excessive level of reactive oxygen species (ROS), which has been related to BPA exposure (Barbonetti et al., 2016; Kaur et al., 2018; Yin et al., 2017). The high degree of DNA fragmentation observed after treatment with 2000 μg/L BPA would overcome the repairing ability of the zygote after fertilization (Fernández-Díez et al., 2018), and could be responsible for promoting embryo death during development.

In conclusion, our study highlights the fact that male exposure to high BPA doses during spermatogenesis induces apoptosis in testicular cells, impairs meiotic progress, missregulates gene expression and promotes epigenetic changes leading to histone hyperacetylation and DNA hypermethylation. Some of these events, particularly changes in histone acetylation, seem to be mediated via GPER upregulation. The promoted testicular alterations affect spermatic transcripts, increase sperm DNA fragmentation and change epigenetic landscape in spermatozoa. Doses of 100 μg/L of BPA, considered safe for water consumption until recent times, and occasionally reported in surface waters and effluents, also modify spermatic epigenetic pattern, compromising forthcoming progeny development upon fertilization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.01.127.

Funding

This work was supported by the Spanish Ministry of Economy and Competitiveness (project AGL2014-53167-C3-3-R), Junta de Castilla y León (Spain) (EDU/1083/2013), the Fondo Social Europeo and by an EMBO Short-Term Fellowship to SGR.

References

Acconcia, F., Pallottini, V., Marino, M., 2015. Molecular mechanisms of action of BPA. Dose. Response. 13, 1559325815610582.

Adoamnei, E., Mendiola, J., Vela-Soria, F., Fernández, M.F., Olea, N., Jørgensen, N., Swan, S.H., Torres-Cantero, A.M., 2018. Urinary bisphenol A concentrations are associated with reproductive parameters in young men. Environ. Res. 161, 122–128.

Ali, S., Steinmetz, G., Montillet, G., Perrard, M.-H., Loundou, A., Durand, P., Guichaoua, M.-R., Prat, O., 2014. Exposure to low-dose bisphenol A impairs meiosis in the rat seminiferous tubule culture model: a physiotoxicogenomic approach. PLoS One 9, e106245.

Avendaño, C., Franchi, A., Jones, E., Oehninger, S., 2009. Pregnancy-specific {beta}-1-glycoprotein 1 and human leukocyte antigen-E mRNA in human sperm: differential expression in fertile and infertile men and evidence of a possible functional role during early development. Hum. Reprod. 24, 270–277.

Bansal, A., Rashid, C., Xin, F., Li, C., Polyak, E., Duemler, A., van der Meer, T., Stefaniak, M., Wajid, S., Doliba, N., Bartolomei, M.S., Simmons, R.A., 2017. Sex- and dose-specific effects of maternal bisphenol A exposure on pancreatic islets of first- and second-generation adult mice offspring. Environ. Health Perspect. 125, 097022.

Barbonetti, A., Castellini, C., Di Giammarco, N., Santilli, G., Francavilla, S., Francavilla, F., 2016. In vitro exposure of human spermatozoa to bisphenol A induces pro-oxidative/apoptotic mitochondrial dysfunction. Reprod. Toxicol. 66, 61–67.

Berger, A., Ziv-Gal, A., Cudiamat, J., Wang, W., Zhou, C., Flaws, J.A., 2016. The effects of in utero bisphenol A exposure on the ovaries in multiple generations of mice. Reprod. Toxicol. 60, 39–52.

Bhandari, R.K., Deem, S.L., Holliday, D.K., Jandegian, C.M., Kassotis, C.D., Nagel, S.C., Tillitt, D.E., vom Saal, F.S., Rosenfeld, C.S., 2015. Effects of the environmental estrogenic contaminants bisphenol A and 17α-ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species. Gen. Comp. Endocrinol. 214, 195–219.

Brykczynska, U., Hisano, M., Erkek, S., Ramos, L., Oakeley, E.J., Roloff, T.C., Beisel, C., Schübeler, D., Stadler, M.B., Peters, A.H.F.M., 2010. Repressive and active histone methylation mark distinct promoters in human and mouse spermatozoa. Nat. Struct. Mol. Biol. 17, 679–687.

Calafat, A.M., Ye, X., Wong, L.-Y., Reidy, J.A., Needham, L.L., 2008. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ. Health Perspect. 116, 39–44.

Carmichael, C., Westerfield, M., Varga, Z.M., 2009. Cryopreservation and in vitro fertilization at the zebrafish international resource center. Methods Mol. Biol. 546, 45–65.

Champroux, A., Cocquet, J., Henry-Berger, J., Drevet, J.R., Kocer, A., 2018. A decade of exploring the mammalian sperm epigenome: paternal epigenetic and transgenerational inheritance. Front. cell Dev. Biol. 6, 50.

Chandrasekar, G., Archer, A., Gustafsson, , Andersson Lendahl, M., 2010. Levels of 17β-estradiol receptors expressed in embryonic and adult zebrafish following in vivo treatment of natural or synthetic ligands. PLoS One 5, e9678.

Chen, J., Xiao, Y., Gai, Z., Li, R., Zhu, Z., Bai, C., Tanguay, R.L., Xu, X., Huang, C., Dong, Q., 2015. Reproductive toxicity of low level bisphenol A exposures in a two-generation zebrafish assay: evidence of male-specific effects. Aquat. Toxicol. 169, 204–214.

Chen, Z., Zuo, X., He, D., Ding, S., Xu, F., Yang, H., Jin, X., Fan, Y., Ying, L., Tian, C., Ying, C., 2017. Long-term exposure to a "safe" dose of bisphenol A reduced protein acetylation in adult rat testes. Sci. Rep. 7, 40337.

Chianese, R., Troisi, J., Richards, S., Scafuro, M., Fasano, S., Guida, M., Pierantoni, R., Meccariello, R., 2017. Bisphenol A in reproduction: epigenetic effects. Curr. Med. Chem. 24, 748–770.

Chimento, A., Sirianni, R., Casaburi, I., Pezzi, V., 2014. GPER signaling in spermatogenesis and testicular tumors. Front. Endocrinol. 5, 30.

Chimento, A., Sirianni, R., Delalande, C., Silandre, D., Bois, C., Andò, S., Maggiolini, M., Carreau, S., Pezzi, V., 2010. 17β-Estradiol activates rapid signaling pathways involved in rat pachytene spermatocytes apoptosis through GPR30 and ERα. Mol. Cell. Endocrinol. 320, 136–144.

Chimento, A., Sirianni, R., Zolea, F., Bois, C., Delalande, C., Andò, S., Maggiolini, M., Aquila, S., Carreau, S., Pezzi, V., 2011. Gper and ESRs are expressed in rat round spermatids and mediate oestrogen-dependent rapid pathways modulating expression of cyclin B1 and Bax. Int. J. Androl. 34, 420–429.

Dagdemir, A., Durif, J., Ngollo, M., Bignon, Y.-J., Bernard-Gallon, D., 2013. Histone lysine trimethylation or acetylation can be modulated by phytoestrogen, estrogen or anti-HDAC in breast cancer cell lines. Epigenomics 5, 51–63.

Dere, E., Anderson, L.M., Huse, S.M., Spade, D.J., McDonnell-Clark, E., Madnick, S.J., Hall, S.J., Camacho, L., Lewis, S.M., Vanlandingham, M.M., Boekelheide, K., 2018. Effects of continuous bisphenol A exposure from early gestation on 90 day old rat testes function and sperm molecular profiles: a CLARITY-BPA consortium study. Toxicol. Appl. Pharmacol. 347, 1–9.

Dong, X., Zhang, Z., Meng, S., Pan, C., Yang, M., Wu, X., Yang, L., Xu, H., 2018. Parental exposure to bisphenol A and its analogs influences zebrafish offspring immunity. Sci. Total Environ. 610–611, 291–297.

Drobná, Z., Henriksen, A.D., Wolstenholme, J.T., Montiel, C., Lambeth, P.S., Shang, S., Harris, E.P., Zhou, C., Flaws, J.A., Adli, M., Rissman, E.F., 2018. Transgenerational effects of bisphenol A on gene expression and DNA methylation of imprinted genes in brain. Endocrinology 159, 132–144.

Fang, Z., Zhu, Q., Gu, T., Shen, X., Yang, Y., Liang, Y., Zhang, Z., Xu, X., 2017. Anti-androgenic effects of bisphenol-A on spatial memory and synaptic plasticity of the hippocampus in mice. Horm. Behav. 93, 151–158.

Feldman, R.D., Limbird, L.E., 2017. GPER (GPR30): a nongenomic receptor (GPCR) for steroid hormones with implications for cardiovascular disease and cancer. Annu. Rev. Pharmacol. Toxicol. 57, 567–584.

Fénichel, P., Chevalier, N., 2017. Environmental endocrine disruptors: new diabetogens?. C. R. Biol. 340, 446–452.

Fernández-Díez, C., González-Rojo, S., Lombó, M., Herráez, M.P., 2018. Tolerance to paternal genotoxic damage promotes survival during embryo development in zebrafish (Danio rerio). Biol. Open 7, bio030130.

Fudala-Ksiazek, S., Pierpaoli, M., Luczkiewicz, A., 2017. Fate and significance of phthalates and bisphenol A in liquid by-products generated during municipal solid waste mechanical-biological pre-treatment and disposal. Waste Manag. 64, 28–38.

Gallo-Oller, G., Ordoñez, R., Dotor, J., 2018. A new background subtraction method for Western blot densitometry band quantification through image analysis software. J. Immunol. Methods 457, 1–5.

Gong, X., Xie, H., Li, X., Wu, J., Lin, Y., 2017. Bisphenol A induced apoptosis and transcriptome differences of spermatogonial stem cells in vitro. Acta Biochim. Biophys. Sin. 49, 780–791.

Hamatani, T., 2012. Human spermatozoal RNAs. Fertil. Steril. 97, 275–281.

Hatef, A., Alavi, S.M.H., Abdulfatah, A., Fontaine, P., Rodina, M., Linhart, O., 2012. Adverse effects of bisphenol A on reproductive physiology in male goldfish at environmentally relevant concentrations. Ecotoxicol. Environ. Saf. 76, 56–62.
Haubruge, E., Petit, F., Gage, M.J.G., 2000. Reduced sperm counts in guppies (Poecilia reticulata) following exposure to low levels of tributyltin and bisphenol A. Proc. R. Soc. B Biol. Sci. 267, 2333–2337.
Hazzouri, M., Pivot-Pajot, C., Faure, A.K., Usson, Y., Pelletier, R., Sèle, B., Khochbin, S., Rousseaux, S., 2000. Regulated hyperacetylation of core histones during mouse spermatogenesis: involvement of histone-deacetylases. Eur. J. Cell Biol. 79, 950–960.
Herráez, M.P., Ausió, J., Devaux, A., González-Rojo, S., Fernández-Díez, C., Bony, S., Saperas, N., Robles, V., 2017. Paternal Contribution to Development: Sperm Genetic Damage and Repair in Fish, vol 472, Aquaculture.
Herz, C., Tran, H.T.T., Schlotz, N., Michels, K., Lamy, E., 2017. Low-dose levels of bisphenol A inhibit telomerase via ER/GPR30-ERK signalling, impair DNA integrity and reduce cell proliferation in primary PBMC. Sci. Rep. 7, 16631.
Hong, J., Chen, F., Wang, X., Bai, Y., Zhou, R., Li, Y., Chen, L., 2016. Exposure of preimplantation embryos to low-dose bisphenol A impairs testes development and suppresses histone acetylation of StAR promoter to reduce production of testosterone in mice. Mol. Cell. Endocrinol. 427, 101–111.
Johnson, G.D., Lalancette, C., Linnemann, A.K., Leduc, F., Boissonneault, G., Krawetz, S.A., 2011. The sperm nucleus: chromatin, RNA, and the nuclear matrix. Reproduction 141, 21–36.
Kaur, S., Saluja, M., Bansal, M.P., 2018. Bisphenol A induced oxidative stress and apoptosis in mice testes: modulation by selenium. Andrologia 50, e12834.
Klosin, A., Casas, E., Hidalgo-Carcedo, C., Vavouri, T., Lehner, B., 2017. Transgenerational transmission of environmental information in C. elegans. Science 356, 320–323.
Lahnsteiner, F., Berger, B., Kletzl, M., Weismann, T., 2005. Effect of bisphenol A on maturation and quality of semen and eggs in the brown trout, Salmo trutta f. fario. Aquat. Toxicol. 75, 213–224.
Laing, L.V., Viana, J., Dempster, E.L., Trznadel, M., Trunkfield, L.A., Uren Webster, T.M., van Aerle, R., Paull, G.C., Wilson, R.J., Mill, J., Santos, E.M., 2016. Bisphenol A causes reproductive toxicity, decreases dnmt1 transcription, and reduces global DNA methylation in breeding zebrafish (Danio rerio). Epigenetics 11, 526–538.
Leal, M.C., de Waal, P.P., García-López, , Chen, S.X., Bogerd, J., Schulz, R.W., 2009. Zebrafish primary testis tissue culture: an approach to study testis function ex vivo. Gen. Comp. Endocrinol. 162, 134–138.
Li, D.-K., Zhou, Z., Miao, M., He, Y., Qing, D., Wu, T., Wang, J., Weng, X., Ferber, J., Herrinton, L.J., Zhu, Q., Gao, E., Yuan, W., 2010. Relationship between urine bisphenol-A level and declining male sexual function. J. Androl. 31, 500–506.
Li, D.-K., Zhou, Z., Miao, M., He, Y., Wang, J., Ferber, J., Herrinton, L.J., Gao, E., Yuan, W., 2011. Urine bisphenol-A (BPA) level in relation to semen quality. Fertil. Steril. 95, 625–630, e4.
Li, Y.-J., Song, T.-B., Cai, Y.-Y., Zhou, J.-S., Song, X., Zhao, X., Wu, X.-L., 2009. Bisphenol A exposure induces apoptosis and upregulation of Fas/FasL and caspase-3 expression in the testes of mice. Toxicol. Sci. 108, 427–436.
Liu, B., Zhou, S., Yang, C., Chen, P., Chen, P., Xi, D., Zhu, H., Gao, Y., 2017. Bisphenol A deteriorates egg quality through HDAC7 suppression. Oncotarget 8, 92359–92365.
Liu, C., Duan, W., Li, R., Xu, S., Zhang, L., Chen, C., He, M., Lu, Y., Wu, H., Pi, H., Luo, X., Zhang, Y., Zhong, M., Yu, Z., Zhou, Z., 2013. Exposure to bisphenol A disrupts meiotic progression during spermatogenesis in adult rats through estrogen-like activity. Cell Death Dis. 4, e676–e676.
Liu, C., Duan, W., Zhang, L., Xu, S., Li, R., Chen, C., He, M., Lu, Y., Wu, H., Yu, Z., Zhou, Z., 2014a. Bisphenol A exposure at an environmentally relevant dose induces meiotic abnormalities in adult male rats. Cell Tissue Res. 355, 223–232.
Liu, Y., Yuan, C., Chen, S., Zheng, Y., Zhang, Y., Gao, J., Wang, Z., 2014b. Global and cyp19a1a gene specific DNA methylation in gonads of adult rare minnow Gobiocypris rarus under bisphenol A exposure. Aquat. Toxicol. 156, 10–16.
Liu, Y., Zhang, Y., Tao, S., Guan, Y., Zhang, T., Wang, Z., 2016. Global DNA methylation in gonads of adult zebrafish Danio rerio under bisphenol A exposure. Ecotoxicol. Environ. Saf. 130, 124–132.
Lombó, M., Fernández-Díez, C., González-Rojo, S., Navarro, C., Robles, V., Herráez, M.P., 2015. Transgenerational inheritance of heart disorders caused by paternal bisphenol A exposure. Environ. Pollut. 206.
MacKay, H., Abizaid, A., 2018. A plurality of molecular targets: the receptor ecosystem for bisphenol-A (BPA). Horm. Behav. 101, 59–67.
Manfo, F.P.T., Jubendradass, R., Nantia, E.A., Moundipa, P.F., Mathur, P.P., 2014. Adverse Effects of bisphenol A on male reproductive function. In: Reviews of Environmental Contamination and Toxicology. pp. 57–82.
Mao, Z., Xia, W., Chang, H., Huo, W., Li, Y., Xu, S., 2015. Paternal BPA exposure in early life alters Igf2 epigenetic status in sperm and induces pancreatic impairment in rat offspring. Toxicol. Lett. 238, 30–38.
Martin, E.M., Fry, R.C., 2018. Environmental influences on the epigenome: exposure-associated DNA methylation in human populations. Annu. Rev. Public Health 39, 309–333.
Meeker, J.D., Ehrlich, S., Toth, T.L., Wright, D.L., Calafat, A.M., Trisini, A.T., Ye, X., Hauser, R., 2010a. Semen quality and sperm DNA damage in relation to urinary bisphenol A among men from an infertility clinic. Reprod. Toxicol. 30, 532–539.
Meeker, J.D., Yang, T., Ye, X., Calafat, A.M., Hauser, R., 2010b. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ. Health Perspect. 119, 252–257.
Morin, N., Arp, H.P.H., Hale, S.E., 2015. Bisphenol A in solid waste materials, leachate water, and air particles from Norwegian waste-handling facilities: presence and partitioning behavior. Environ. Sci. Technol. 49, 7675–7683.
Murata, M., Kang, J.-H., 2018. Bisphenol A (BPA) and cell signaling pathways. Biotechnol. Adv. 36, 311–327.
Okada, H., Tokunaga, T., Liu, X., Takayanagi, S., Matsushima, A., Shimohigashi, Y., 2007. Direct evidence revealing structural elements essential for the high binding ability of bisphenol A to human estrogen-related receptor-γ. Environ. Health Perspect. 116, 32–38.
Ostermeier, G.C., Miller, D., Huntriss, J.D., Diamond, M.P., Krawetz, S.A., 2004. Reproductive biology: delivering spermatozoan RNA to the oocyte. Nature 429, 154.
Pariante, P., Dotolo, R., Venditti, M., Ferrara, D., Donizetti, A., Aniello, F., Minucci, S., 2016. Prothymosin alpha expression and localization during the spermatogenesis of Danio rerio. Zygote 24, 583–593.
Peretz, J., Vrooman, L., Ricke, W.A., Hunt, P.A., Ehrlich, S., Hauser, R., Padmanabhan, V., Taylor, H.S., Swan, S.H., VandeVoort, C.A., Flaws, J.A., 2014. Bisphenol A and reproductive health: update of experimental and human evidence, 2007-2013. Environ. Health Perspect. 122, 775–786.
Pérez-Cerezales, S., Ramos-Ibeas, P., Lopez-Cardona, A., Pericuesta, E., Fernandez-Gonzalez, R., Pintado, B., Gutiérrez-Adán, A., 2017. Elimination of methylation marks at lysines 4 and 9 of histone 3 (H3K4 and H3K9) of spermatozoa alters offspring phenotype. Reprod. Fertil. Dev. 29, 740.
Prossnitz, E.R., Arterburn, J.B., 2015. International Union of Basic and Clinical Pharmacology. XCVII. G protein-coupled estrogen receptor and its pharmacologic modulators. Pharmacol. Rev. 67, 505–540.
Prossnitz, E.R., Barton, M., 2011. The G-protein-coupled estrogen receptor GPER in health and disease. Nat. Rev. Endocrinol. 7, 715–726.
Prossnitz, E.R., Sklar, L.A., Oprea, T.I., Arterburn, J.B., 2008. GPR30: a novel therapeutic target in estrogen-related disease. Trends Pharmacol. Sci. 29, 116–123.
Qian, W., Wang, Y., Zhu, J., Mao, C., Wang, Q., Huan, F., Cheng, J., Liu, Y., Wang, J., Xiao, H., 2015. The toxic effects of bisphenol A on the mouse spermatocyte GC-2 cell line: the role of the Ca^{2+}-calmodulin-Ca^{2+}/calmodulin-dependent protein kinase II axis. J. Appl. Toxicol. 35, 1271–1277.
Rahman, M.S., Kwon, W.-S., Karmakar, P.C., Yoon, S.-J., Ryu, B.-Y., Pang, M.-G., 2017. Gestational exposure to bisphenol A affects the function and proteome profile of F1 spermatozoa in adult mice. Environ. Health Perspect. 125, 238–245.
Rubin, B.S., 2011. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J. Steroid Biochem. Mol. Biol. 127, 27–34.
Schagdarsurengin, U., Steger, K., 2016. Epigenetics in male reproduction: effect of paternal diet on sperm quality and offspring health. Nat. Rev. Urol. 13, 584–595.
Schulz, R.W., de França, L.R., Lareyre, J.-J., LeGac, F., Chiarini-Garcia, H., Nobrega, R.H., Miura, T., Miura, T., 2010. Spermatogenesis in fish. Gen. Comp. Endocrinol. 165, 390–411.
Shechter, D., Dormann, H.L., Allis, C.D., Hake, S.B., 2007. Extraction, purification and analysis of histones. Nat. Protoc. 2, 1445–1457.
Shi, M., Sekulovski, N., MacLean, J.A., Hayashi, K., 2017. Effects of bisphenol A analogues on reproductive functions in mice. Reprod. Toxicol. 73, 280–291.
Sun, H., Xu, L.-C., Chen, J.-F., Song, L., Wang, X.-R., 2006. Effect of bisphenol A, tetrachlorobisphenol A and pentachlorophenol on the transcriptional activities of androgen receptor-mediated reporter gene. Food Chem. Toxicol. 44, 1916–1921.
Swann, K., Lai, F.A., 2016. Egg activation at fertilization by a soluble sperm protein. Physiol. Rev. 96, 127–149.
Thomas, P., 2017. Role of G-protein-coupled estrogen receptor (GPER/GPR30) in maintenance of meiotic arrest in fish oocytes. J. Steroid Biochem. Mol. Biol. 167, 153–161.
Thomas, P., Dong, J., 2006. Binding and activation of the seven-transmembrane estrogen receptor GPR30 by environmental estrogens: a potential novel mechanism of endocrine disruption. J. Steroid Biochem. Mol. Biol. 102, 175–179.
Valcarce, D.G., Vuelta, E., Robles, V., Herráez, M.P., 2017. Paternal exposure to environmental 17-alpha-ethinylestradiol concentrations modifies testicular transcription, affecting the sperm transcript content and the offspring performance in zebrafish. Aquat. Toxicol. 193, 18–29.
Wang, C., Zhang, J., Li, Q., Zhang, T., Deng, Z., Lian, J., Jia, D., Li, R., Zheng, T., Ding, X., Yang, F., Ma, C., Wang, R., Zhang, W., Wen, J.G.,

2017a. Low concentration of BPA induces mice spermatocytes apoptosis via GPR30. Oncotarget 8, 49005–49015.
Wang, H., Ding, Z., Shi, Q.-M., Ge, X., Wang, H.-X., Li, M.-X., Chen, G., Wang, Q., Ju, Q., Zhang, J.-P., Zhang, M.-R., Xu, L.-C., 2017b. Anti-androgenic mechanisms of bisphenol A involve androgen receptor signaling pathway. Toxicology 387, 10–16.
Wang, P., Luo, C., Li, Q., Chen, S., Hu, Y., 2014. Mitochondrion-mediated apoptosis is involved in reproductive damage caused by BPA in male rats. Environ. Toxicol. Pharmacol. 38, 1025–1033.
Wang, Q., Zhao, X.-F., Ji, Y.-L., Wang, H., Liu, P., Zhang, C., Zhang, Y., Xu, D.-X., 2010. Mitochondrial signaling pathway is also involved in bisphenol A induced germ cell apoptosis in testes. Toxicol. Lett. 199, 129–135.
Willhite, C.C., Ball, G.L., McLellan, C.J., 2008. Derivation of a bisphenol A oral reference dose (RfD) and drinking-water equivalent concentration∗. J. Toxicol. Environ. Health Part B 11, 69–146.
Xie, M., Bu, P., Li, F., Lan, S., Wu, H., Yuan, L., Wang, Y., 2016. Neonatal bisphenol A exposure induces meiotic arrest and apoptosis of spermatogenic cells. Oncotarget 7, 10606–10615.
Xin, F., Susiarjo, M., Bartolomei, M.S., 2015. Multigenerational and transgenerational effects of endocrine disrupting chemicals: a role for altered epigenetic regulation?. Semin. Cell Dev. Biol. 43, 66–75.
Yamauchi, Y., Shaman, J.A., Ward, W.S., 2011. Non-genetic contributions of the sperm nucleus to embryonic development. Asian J. Androl. 13, 31–35.
Yin, L., Dai, Y., Cui, Z., Jiang, X., Liu, W., Han, F., Lin, A., Cao, J., Liu, J., 2017. The regulation of cellular apoptosis by the ROS-triggered PERK/EIF2α/chop pathway plays a vital role in bisphenol A-induced male reproductive toxicity. Toxicol. Appl. Pharmacol. 314, 98–108.
Yuan, C., Zhang, Y., Liu, Y., Wang, S., Wang, Z., 2017. DNA demethylation mediated by down-regulated TETs in the testes of rare minnow Gobiocypris rarus under bisphenol A exposure. Chemosphere 171, 355–361.
Yuan, C., Zhang, Y., Liu, Y., Zhang, T., Wang, Z., 2016. Enhanced GSH synthesis by Bisphenol A exposure promoted DNA methylation process in the testes of adult rare minnow Gobiocypris rarus. Aquat. Toxicol. 178, 99–105.
Zhang, G.-L., Zhang, X.-F., Feng, Y.-M., Li, L., Huynh, E., Sun, X.-F., Sun, Z.-Y., Shen, W., 2013. Exposure to bisphenol A results in a decline in mouse spermatogenesis. Reprod. Fertil. Dev. 25, 847.
Zhang, T., Liu, Y., Chen, H., Gao, J., Zhang, Y., Yuan, C., Wang, Z., 2017. The DNA methylation status alteration of two steroidogenic genes in gonads of rare minnow after bisphenol A exposure. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 198, 9–18.
Zhang, Y., Cheng, M., Wu, L., Zhang, G., Wang, Z., 2016. Bisphenol A induces spermatocyte apoptosis in rare minnow Gobiocypris rarus. Aquat. Toxicol. 179, 18–26.
Zheng, H., Zhou, X., Li, D., Yang, F., Pan, H., Li, T., Miao, M., Li, R., Yuan, W., 2017. Genome-wide alteration in DNA hydroxymethylation in the sperm from bisphenol A-exposed men. PLoS One 12, e0178535.

ETOC BLURB

Male BPA exposure impairs spermatogenesis and alters the epigenetic pattern of testicular cells, leading to hyperacetylation of histones and DNA hypermethylation, which can promote adverse effects on the zygote upon fertilization.