Full length article

Abnormal histone replacement following BPA exposure affects spermatogenesis and fertility sequentially

Do-Yeal Ryu , Won-Ki Pang , Elikanah Olusayo Adegoke , Md Saidur Rahman , Yoo-Jin Park , Myung-Geol Pang *

Department of Animal Science & Technology and BET Research Institute, Chung-Ang University, Anseong, Gyeonggi-do 17546, Republic of Korea

ABSTRACT

Bisphenol A (BPA) is an endocrine-disrupting chemical widely distributed in the environment. Its exposure has been linked to male infertility in animals and humans due to its ability to induce epigenetic modification. Despite extensive research confirming the impact of BPA on epigenetic regulation, fundamental concerns about how BPA causes epigenetic changes and the underlying mechanism of BPA on the male reproductive system remain un-resolved. Therefore, we sought to investigate the effects of BPA on epigenetic regulation and the histone-to-protamine (PRM) transition, which is fundamental process for male fertility in testes and spermatozoa by exposing male mice to BPA for 6 weeks while giving the mice in the control group corn oil by oral gavage. Our results demonstrated that the mRNA levels of the histone family and PRMs were significantly altered by BPA exposure in testes and spermatozoa. Subsequently, core histone proteins, the PRM1/PRM2 ratio, directly linked to male fertility, and transition proteins were significantly reduced. Furthermore, we discovered that BPA significantly caused abnormal histone-to-protamine replacement during spermiogenesis by increased histone variants-related to histone-to-PRM transition. The levels of histone H3 modification in the testes and DNA methylation in spermatozoa were significantly increased. Consequently, sperm concentration/motility/hyper-activation, fertilization, and early embryonic development were adversely affected as a consequence of altered signaling proteins following BPA exposure. To our knowledge, this is the first study to indicate that BPA exposure influences the histone-to-PRM transition via altering epigenetic modification and eventually causing reduced male fertility.

1. Introduction

Endocrine-disrupting chemicals (EDCs) are well known to have detrimental effects on neurological, cardiovascular, developmental, metabolic, and reproductive physiology in humans (Schug et al., 2011). Bisphenol A (BPA) is one of the most prevalent EDCs due to its diverse uses (Ikezuki et al., 2002; Welshons et al., 2006). BPA is broadly used in products produced by the plastics industry, including mineral water bottles, toys, food and beverage packaging materials, and dental filling materials (Hope et al., 2016; Kubwabo et al., 2009; Rahman et al., 2021a). As a result, BPA exposure is widespread, with human studies indicating its presence in more than 90% of urine samples in the United States (Bao et al., 2020; Calafat et al., 2008; Rahman et al., 2017). BPA can disrupt or mimic hormone action by binding to a range of physiological receptors, including estrogen/androgen receptors, due to its estrogen-like structures (Acerini and Hughes, 2006; Schug et al., 2011).

In recent decades, several studies have shown that BPA is linked to the etiology of various chronic diseases, such as obesity, diabetes, cardiovascular disease, developmental abnormalities, and reproductive diseases via mitochondrial dysfunctions, abnormal endocrine systems, oxidative stress, and genetic and epigenetic changes (Park et al., 2021; Park et al., 2020; Rezg et al., 2014; Rochester, 2013; Ryu et al., 2017b). Like most EDCs, BPA is reported to exert epigenetic effects (Chianese et al., 2018; Rahman et al., 2020).

Epigenetics is a branch of genetics that investigates how environmental variables cause genetic changes without altering the DNA sequence. Non-coding RNA expression, chromatin remodeling, DNA methylation, and histone modifications are examples of epigenetic changes that can affect the phenotype of different cell types (Dolinoy and Jirtle, 2008). Furthermore, epigenetic mechanisms play a crucial role in modulating environmental stress responses, thereby influence the normal development and cause disorders (Anway et al., 2008). Recent

evidence shows that BPA exposure alters the normal epigenetic patterns in the male reproductive system (Chianese et al., 2018; Song et al., 2019). However, the varied effects of BPA in spermatogonia depending on the BPA dose indicate that complex underlying mechanisms are involved.

In vitro exposure of mice germ cells to high doses of BPA (1 and 10 μg/ml) but not lower doses (0.001–0.1 μg/ml) decreased the global levels of DNA methylation and H3K9me3 in spermatogonial cells, disrupting spermatogenesis due to a reduction in the mRNA and protein levels of DNA methyltransferase, while the low doses increased the levels of H3K9me3 and H3K27me (Li et al., 2018). Decreased di-methylation of H3K9 was involved in the adverse effects on spermatogenesis following in vitro exposure of prepubertal mouse testes to BPA (Zhang et al., 2018). Similar observations have been reported in humans. Human sperm motility, concentration, and mitochondrial function were negatively correlated with H3K4me2 (Stiavnicka et al., 2020), and reduced sperm concentration and motility induced by BPA exposure were linked to DNA hydroxymethylation due to histone H3 trimethylation (Zheng et al., 2017). Furthermore, at the highest dose of BPA (50 mg BPA/kg body weight [BW]/day), our previous investigations found that early-life BPA exposure caused histone modification and DNA methylation in spermatozoa, resulting in transgenerational and multigenerational effects on offspring phenotypes and their fertility (Rahman et al., 2020; Rahman et al., 2021b).

Mammalian spermatozoa are compartmental cells with a compact nucleus, in which the DNA is tightly condensed by protamines (PRMs) ((Oliva and Ballesca, 2012; Oliva and Dixon, 1991). PRMs are arginine-rich, nuclear proteins with two major forms, PRM1 and PRM2. Spermiogenesis, the last phase of spermatogenesis, changes round spermatids to spermatozoa. During spermiogenesis, maturing male germ cells undergo histone-to-PRM transition, a process critical for testicular development and male fertility. Histones are replaced first by transition proteins (TNPs) and then by PRMs (Steger et al., 1998), which provide extensive DNA compaction, protect the genome from DNA damage, and preserve genetic traits (Schneider et al., 2016). Histone modifications have an important role in replacing histones with PRMs (Francis et al., 2014). Although histone replacement occurs in most of the sperm genome, many regions retain nucleosomal histones, which are enriched in specific epigenetic modifications, such as H3K4me3, H3K27me3, and unmethylated DNA. Human clinical studies have shown that sperm DNA fragmentation is highly correlated with aberrant PRM replacement and the failure of in vitro fertilization (IVF), intracytoplasmic sperm injection, and pregnancy (Avendano et al., 2010; Carlini et al., 2017; Ni et al., 2016; Nili et al., 2009; Wdowiak et al., 2015). These studies suggest that both histone modification and the histone-to-PRM transition may participate in the disruption of male fertility following BPA exposure.

Although male fertility involves complex functional/physiological alterations beyond spermatogenesis, few studies have investigated the effects of BPA exposure on histone modification and the histone-to-PRM transition in the entire male reproductive system. The aim of this study was to determine if BPA exposure causes changes in core histone expression levels and the histone-to-PRM transition, which leads to male infertility, and if so, to uncover potential biomarkers for diagnosing male infertility after BPA exposure. Simultaneously, histone modifications and DNA methylation were evaluated to comprehensively explore the epigenetic alterations in testes and spermatozoa after BPA exposure.

2. Materials and methods

2.1. Ethics statement

All procedures were performed according to the standard guidelines for animal studies and were approved by the Institutional Animal Care and Use Committee (IACUC) of Chung-Ang University, Seoul, Korea (No. a2022016).

2.2. Chemicals, reagents, and media

Unless otherwise specified, all chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). To obtain the target molecular concentration, BPA was dissolved in corn oil. Modified Tyrode's medium was used as the basic medium (BM) for spermatozoa according to a previously published study (Ryu et al., 2017a). Bovine serum albumin (BSA; 4 mg/ml) was added and incubated at 37 °C under 5% CO_2 conditions overnight before the study began.

2.3. Animals and sample collection

Four-week-old CD-1 (ICR) male mice were purchased from Daehan BioLink® (Chungcheongbuk-do, Korea). Mice were housed at 50–60% humidity and 20–25 °C under a 12:12-h light:dark cycle and were provided commercial pellets and water ad libitum. Mice were acclimated to the environment for a week and then divided into control and lowest observed adverse effect level (LOAEL) groups (n = 40 mice/group). A 50 mg/kg BW/day BPA dose was chosen for this study as the LOAEL defined by the U.S. Environmental Protection Agency (Hengstler et al., 2011; Tyl, 2009). Corn oil was orally administered to the control mice. Both groups were gavaged daily from the ages of 6 to 11 weeks (6-week treatment) based on the daily changes in BW. Mice were stabilized for a week before sampling. Afterward, testes and spermatozoa were collected from each mouse. RNAlater (Invitrogen, Carlsbad, CA, USA) was incubated with testes and caput/cauda epididymis overnight at 4 °C and frozen rapidly in liquid nitrogen (−196 °C) for RNA extraction. At the same time, spermatozoa were harvested from cauda, and collected spermatozoa were snap-frozen in liquid nitrogen (−196 °C) for RNA extraction. Simultaneously, all samples were harvested and stored at −80 °C for Western blot.

2.4. RNA extraction, cDNA synthesis, and quantitative real-time PCR (qRT-PCR)

RNA extraction and cDNA synthesis were conducted on according to a previously published study (Pang et al., 2020). PureLink™ RNA Mini Kit (Invitrogen) and TRIzol (Invitrogen) were used for RNA extraction following the manufacturer's instructions. RNA concentration and the 260/280 ratio were assessed using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). cDNA synthesis was conducted using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions. Subsequently, primers were designed using PrimerSelect™ software (DNASTAR, Madison, WI, USA) (Supplementary Table S1). qRT-PCR was performed using an ABI PRISM 7500 real-time PCR system (Applied Biosystems). The relative quantification of gene expression level compared to the control group was evaluated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.5. Histone extraction and Western blot analysis

Testes (n = 3 mice/group) were collected and cut into small (1–2 mm^3) pieces with a scalpel. Chopped samples and spermatozoa were homogenized in Triton extraction buffer (0.5% Triton X-100, 2 mM phenylmethylsulfonyl fluoride, and 0.02% NaN_3 in PBS). Homogenized samples were centrifuged (3,000 rpm, 4 °C, 5 min), and the supernatants were removed. The pellets were resuspended in extraction buffer per 200 mg of testes and 1×10^7 spermatozoa on ice for 30 min. Lysates were centrifuged (12,000 rpm, 4 °C, 5 min), and the supernatant was collected. Acetone was added to the supernatant and incubated at −20 °C overnight. The histone extracts of all samples were then loaded on an SDS polyacrylamide gel and electrophoresed as reported (Park et al., 2020; Ryu et al., 2017a). The separated protein bands were transferred to a polyvinylidene fluoride (PVDF) membrane (Amersham, Piscataway, NJ, USA). The membrane was incubated with a blocking agent (3%;

Amersham) for 1 h at room temperature. Antibodies against histones H1/H2A/H2B/H3/H4, p38, and GPR30 (Abcam, Cambridge, England), PRM1, TNP1 (Novus Biologicals), PRM2 (Thermo Fisher), TNP2 (LSBio), estrogen receptor (ER) α/β, and PI3K (Santa Cruz Biotechnology) were incubated with membranes overnight at 4 °C. Then, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Abcam) for 1 h at room temperature. β-actin (Cell Signaling Technology) was used as an internal control. The membranes were washed with PBS-T three times, and chemiluminescence was applied to detect the labeled antigens. Quantification of each band was performed using ImageJ software (National Institutes of Health [NIH], Bethesda, MD, USA).

2.6. Immunohistochemistry

To determine precisely at what moment BPA affects the expression of core histone and protamine during the spermatogenesis, IHC staining was performed. Testes (n = 3mice/group) were harvested and fixed in Bouin's solution overnight, followed by embedding in paraffin (Hogarth and Griswold, 2013). Three-micron-thick sections were deparaffinized in xylene and rehydrated in ethanol. The antigen retrieval step was performed using a DAKO target retrieval solution (pH 6 and 9; DAKO, Glostrup, Denmark). anti-rabbit histones H1.0, H2A, H2B, H3, and H4 primary antibody (Abcam), anti-rabbit PRM1 primary antibody (Novus Biologicals), and anti-rabbit PRM2 primary antibody (Thermo Fisher) were incubated overnight. Slides were stained with 3,3′-dia-minobenzidine (envision kit; DAKO) and then counterstained with he-matoxylin. Images were captured using a Nikon Eclipse Ni-U equipped with a Nikon DS-Ri2 camera and Nikon NIS-Elements Fr software (Version 5.11; Nikon Instruments, Inc., Melville, NY, USA). Quantifica-tion of each protein was conducted using ImageJ Fiji software (National Institutes of Health [NIH], Bethesda, MD, USA) according to the following protocol (Crowe and Yue, 2019).

2.7. Total histone H3 modification analysis

Total histone H3 modification was evaluated using Epigentek's EpiQuik® Histone H3 Modification Multiplex Assay Kit according to the manufacturer's instructions. In brief, acid extraction of histones was performed, and the extracts were stably spotted on strip wells. The an-tigens bound to antibodies were calorimetrically analyzed at 450 nm and quantified using a standard curve.

2.8. Global DNA methylation analysis

DNA methylation was evaluated using a global DNA methylation test kit according to the manufacturer's instructions (Abcam). The gDNA was extracted from the spermatozoa using the procedures described by (Rahman et al., 2020). Then, 100 ng of extracted sperm DNA (260/280 ratio > 1.6) was dissolved in the binding solution and incubated for 1 h at 37 °C. After washing, 50 μL of the 5-methyl cytosine (5-mC) detection complex comprising 5-mC antibodies was dispensed into each well and incubated for 30 min at room temperature before being washed again. Each well was then filled with 100 μL of developer solution. The enzy-matic reaction was stopped by introducing a stop solution based on the color shift in the 5% positive control (PC) wells (deep blue). Finally, the absorbance was measured at 450 nm using a Gemini™ microplate reader (Molecular Devices, San Jose, CA, USA). The global DNA methylation experiment was repeated for all samples, and the average data were used to determine the percentage of 5-mC (for each replica-tion) using the standard curve generated by the PC and negative control, which were run simultaneously in each plate with samples. Under specified exposure settings, the final results of DNA methylation are provided as a percentage of methylated DNA (% 5-mC).

2.9. Computer-assisted sperm analysis (CASA)

Sperm concentration, motility and hyperactivity (HYP) were evalu-ated using the CASA system (SAIS Plus version 10.1; Medical supply, Seoul, Korea). Briefly, Makler chamber (Makler, Haifa, Israel) was used, and 10 μL of the sperm suspension (n = 3 mice/group) was placed in it. The chamber was heated to a 37 °C. We used the SAIS software and 10 × phase contrast objective microscope to evaluate sperm motion kine-matics. The CASA parameters were set as addressed previously (frames acquired, 20; frame rate, 30 Hz; minimum contrast, 7; minimum size, 5; low/high size gates, 0.4–1.5; low/high intensity gates, 0.4–1.5; non-motile head size, 16; and non-motile brightness, 14).

2.10. IVF

Female hybrid B6D2F1/CrljOri mice (Nara Biotech, Seoul, Korea) were used for IVF between the ages of eight and twelve weeks (n = 3 mice/group). Mice were given intraperitoneal injections of 5 IU preg-nant mare serum gonadotropin (PMSG) and 5 IU human chorionic gonadotropin (hCG) at 48 h intervals to induce superovulation. Cumulus-oocyte complexes (COCs) were collected from the ampulla and placed in a sterile cell culture dish with DPBS fifteen hours after the hCG injection. The COCs were cultured at 37 °C in 5% CO_2 for 1 h prior to insemination in 50 μL of BM containing 10% fetal bovine serum (FBS) under mineral oil (Ryu et al., 2017a). COCs from were divided evenly between the control and BPA groups. To induce capacitation, sperma-tozoa (n = 3 mice/group) from the cauda epididymis were washed and cultured for 90 min in BM containing 0.4% BSA (Ryu et al., 2017a) and then inseminated (1 × 10^6 cells/ml) using COCs that had been pre-incubated in BM containing 10% fetal bovine serum under mineral oil for 6 h. The number of two-cell embryos formed 18 h after insemination was used to calculate the fertilization rate. Two-cell embryos were cultivated for an additional 4 days in BM with 0.4% BSA under the same conditions. We counted all blastocyst-stage embryos. The final cleavage and blastocyst development results are expressed as a percentage of each parameter under specified exposure settings.

2.11. Statistical analysis

Data were analyzed using a one-way ANOVA with IBM SPSS Statis-tics software (Version 25.0; IBM Corp., Chicago, IL, USA). The Student's two-tailed *t*-test was used to identify significant differences between the means of the control and BPA groups. Differences between the control and BPA groups were considered significant at p-values less than 0.05. Data are presented as mean ± SD.

3. Results

3.1. Impacts of BPA on histone families and PRMs mRNA expression levels in testes and spermatozoa

Six (H2A, H2AC13, H2B, H3C10, H4C8, and H4C12) among ten histone families were significantly altered in the testes of the BPA group (p < 0.05, Fig. 1A). Moreover, seven histone families were altered in the spermatozoa following BPA exposure (p < 0.05, Fig. 1B). Subsequent evaluation of the PRM1/PRM2 ratio revealed that BPA exposure increased the PRM1/PRM2 ratio in both testes and spermatozoa (p < 0.05, Fig. 1).

3.2. Impacts of BPA on core histones, PRMs, and TNPs protein expression levels in testes

To assess the regulatory mechanism of histone-to-PRM transition following BPA exposure, the levels of related proteins (histones H1/H2A/H2B/H3/H4, PRM1/2, and TNP1/2) were measured in testes. All histone families increased following BPA exposure, except for H1 (p <
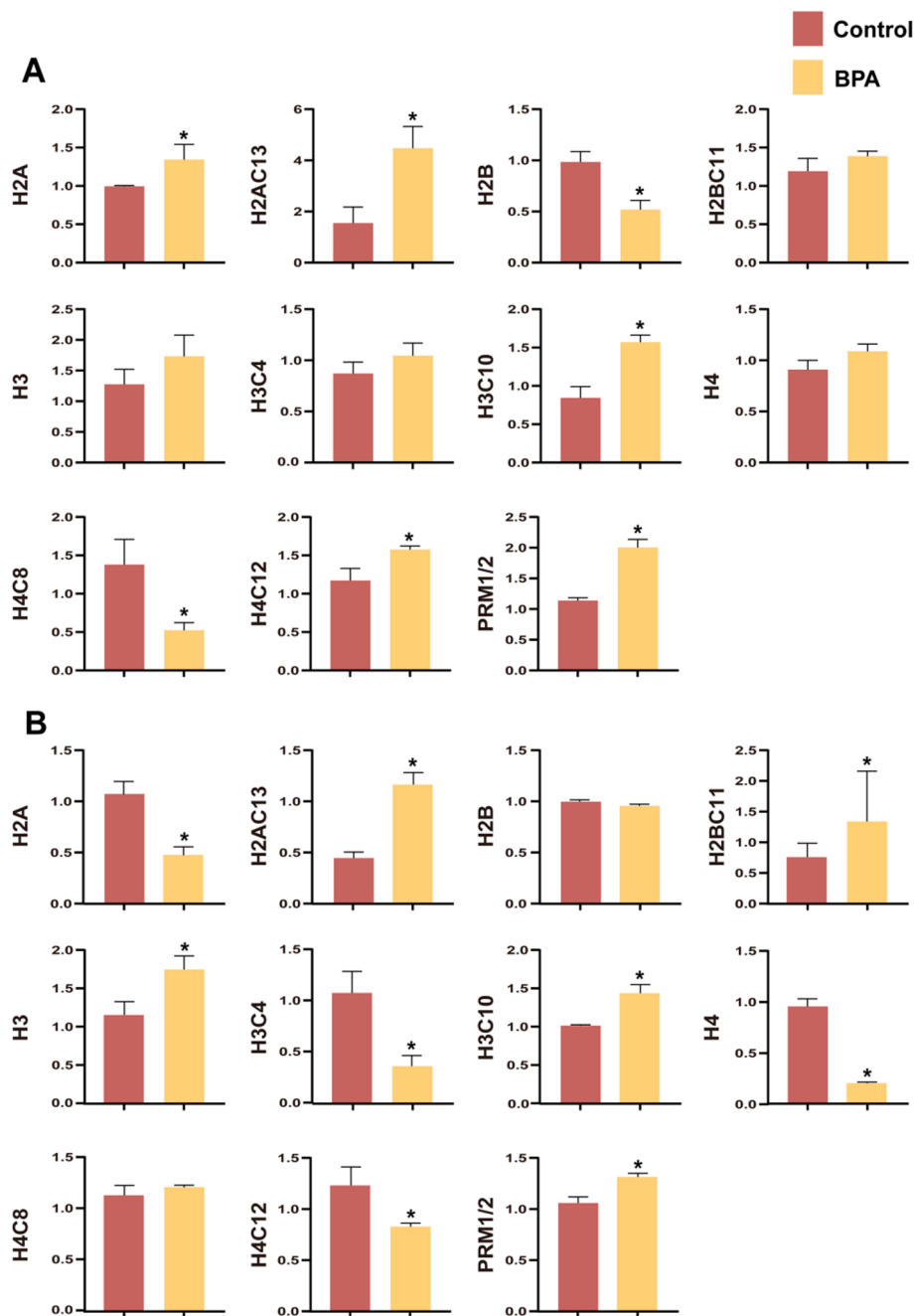
Fig. 1. Difference in levels of histone mRNA expression between control and BPA groups in (**A**) testes and (**B**) spermatozoa. Data are mean of three replicates ± SD. Asterisks denote significant differences between control and treatment groups at p < 0.05 by two-tailed Student's *t*-test.

0.05, Fig. 2A and C). TNP1 decreased significantly in the BPA group, while there was no difference in the TNP2 expression levels between the control and BPA-exposed mice. Subsequent analysis showed that the PRM1/PRM2 ratio decreased in testes following BPA exposure (p < 0.05, Fig. 2A and C) (see Fig. 3).
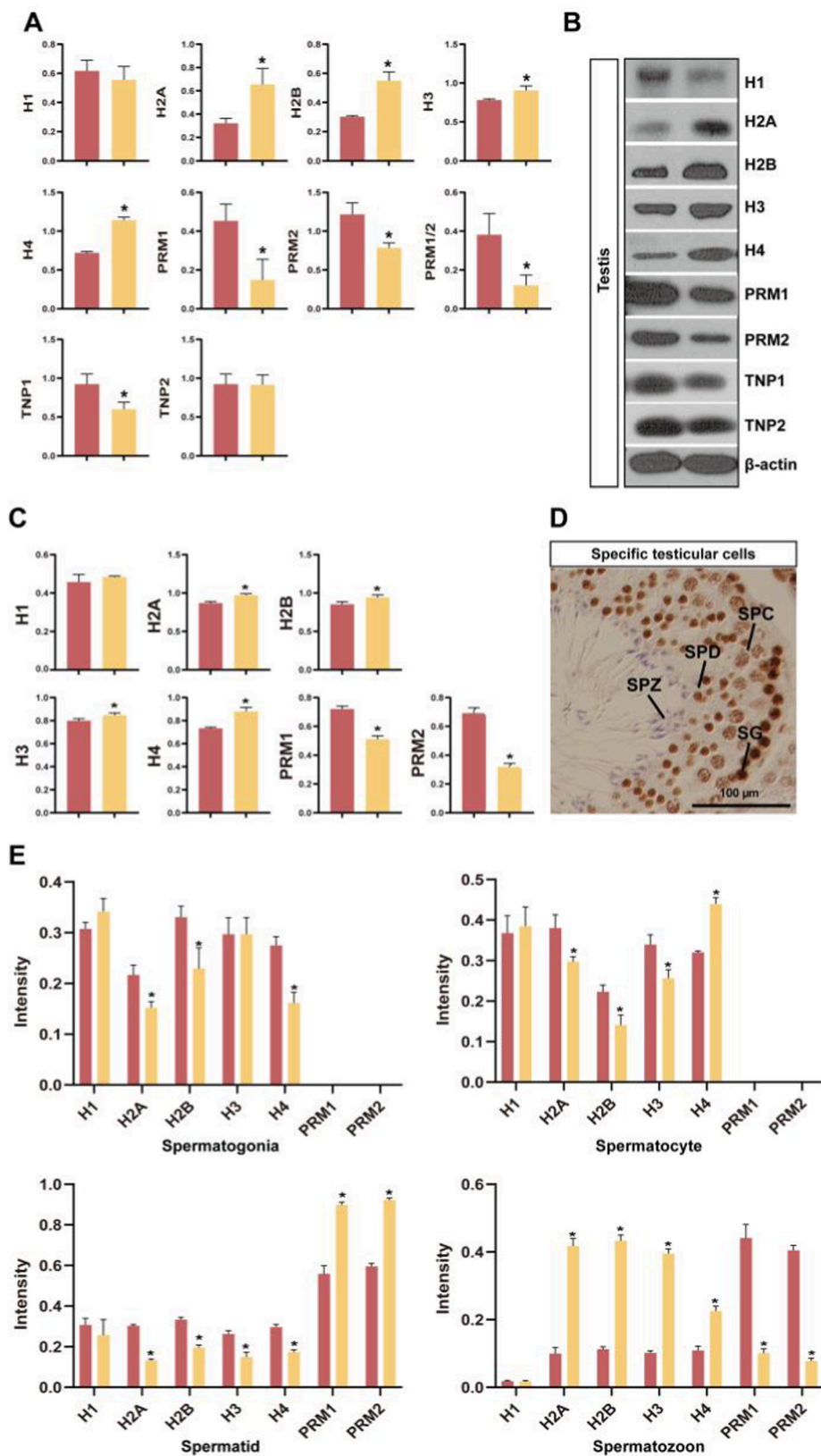
3.3. Impacts of BPA on histone-to-PRM transition-related protein expression levels during spermatogenesis

The expression of each protein was quantified using the ImageJ Fiji program. Our results showed that H2A, H2B, H3, and H4 were significantly lower in the BPA group compared to the control group in spermatogonia, spermatocyte, and spermatid except for H4 in spermatocyte. Interestingly, PRM 1 and 2 were significantly higher in spermatids while

markedly lower in the BPA group in spermatozoon (p < 0.05, Fig. 2E).

3.4. Impacts of BPA on histone variants and histone-to-PRM transition-related genes mRNA expression levels

To elucidate how BPA hinders the histone-to-PRM transition, we evaluated histone variants and cluster genes related to histone-to-PRM transition. H1T2 and H2AL2 were significantly upregulated following BPA exposure among six histone variants (p < 0.05, Fig. 4). In addition, H3F3A was significantly increased among 19 genes related to histone-to-PRM transition (p < 0.05, Fig. 4).

4

Fig. 2. Effects of BPA on protein expression levels during spermatogenesis. (**A**) Density of histone-to-PRM transition-related proteins in testes. (**B**) Representative Western blot image of proteins. (**C**) Intensity of histone-to-PRM transition-related proteins in testes. (**D**) Representative immunohisto-chemistry images of specific testicular cells. Bar = 100 μm. (**E**) Intensity of histone-to-PRM transition-related proteins in different types of germ cells. Data are mean of three replicates ± SD. Asterisks denote significant differences between control and treatment groups at p < 0.05 by two-tailed Student's *t*-test.

3.5. Impacts of BPA on histone H3 modification in testes and global DNA methylation in spermatozoa

ELISA was performed to evaluate the histone H3 modification following BPA exposure. H3K4me1/2/3, H3K9me2/3, H3K27me3, H3K36me1/3, H3K79me1, H3Kac, H3K14ac, H3K56ac, and H3S28P were significantly expressed in testes following BPA exposure (p < 0.05, Fig. 5). We also examined global DNA methylation to gain insights into BPA-mediated epigenetic alterations in sperm function and fertility. Compared to control males, the 5-mC levels in BPA-treated males were
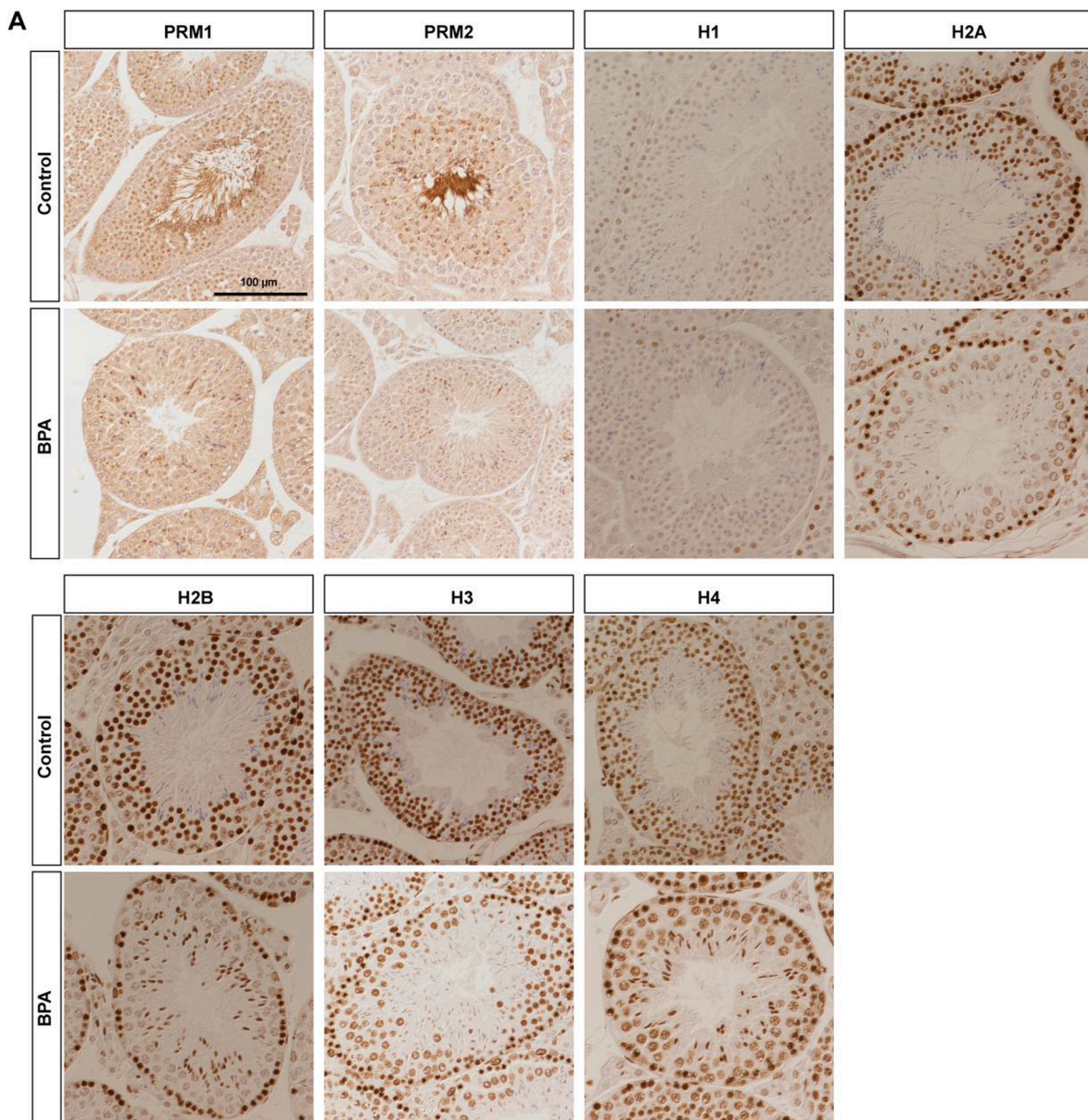
Fig. 3. Representative immunohistochemistry images of core histones and PRMs. Bar = 100 μm.

greater when expressed as a proportion of total DNA (Fig. 5, p < 0.05).

### 3.6. Impacts of BPA on signaling proteins, sperm kinematic parameters and male fertility

We examined the ERs in the testes to determine the underlying mechanism of BPA on testes. BPA decreased the expression of both ERs (α and β), PI3K, and GPR30, while p38 was increased, as shown in Fig. 5E (p < 0.05). Subsequently, we demonstrated that BPA exposure significantly affected sperm concentration, motility, and HYP as a consequence of an altered signaling pathway. Finally, we investigated the fertilizing ability of spermatozoa with oocytes and early embryonic development by an IVF system. BPA exposure decreased the rate of cleavage and blastocyst formation ability of the spermatozoa (p < 0.05, Fig. 5F).

4. Discussion

Our preliminary RNA-sequencing study revealed that BPA influenced the expression levels of several histone families, including H2AC13, H2BC11, H3C4, H3C10, H4C12, and H4C8, in testes and spermatozoa. These RNA-sequencing results were reproduced in the qRT-PCR analysis. Among six histone families, three (H2AC13, H3C10, and H4C12) and two (H2AC13 and H3C10) were significantly increased in testes and spermatozoa of BPA-exposed mice, respectively. H2AC13 and H3C10 have been associated with low-motility sperm via aberrant CpG (Pacheco et al., 2011). In addition, H4C8 was linked to teratozoospermia in humans (Platts et al., 2007). Based on our findings, we hypothesized that the BPA-induced regulation of H2AC13, H3C10, and H4C8 could affect sperm motility and aberrant sperm morphology, leading to male infertility. Although histone family genes play a critical role in most biological systems, little research on male fertility has been undertaken. More research is needed to understand better the role of the histone family genes in male reproduction.

Spermatogenesis, the complex process of sperm production involving consecutive cell differentiation, can be divided into spermatogonia meiosis, spermatocyte meiosis, and spermiogenesis (Hess and Renato de Franca, 2008; Roosen-Runge, 1962). During spermatogenesis,
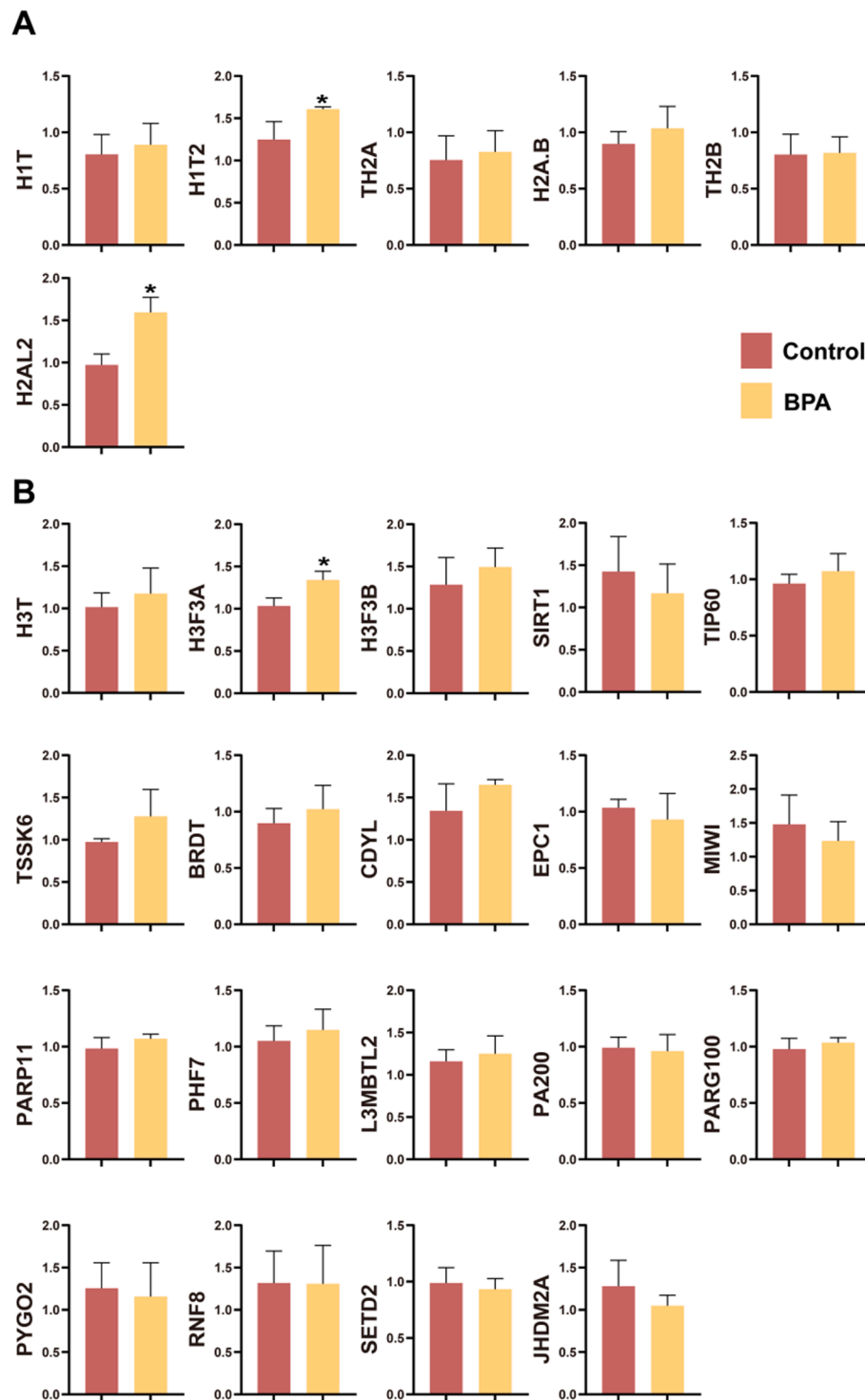
Fig. 4. Difference in levels of (**A**) histone variants and (**B**) genes-related to histone-to-PRM transition between control and BPA groups. Data are mean of three replicates ± SD. Asterisks denote significant differences between control and treatment groups at $p < 0.05$ by two-tailed Student's *t*-test.

spermatogonial stem cells undergo self-renewal and differentiate into spermatocyte and then spermatid (Rathke et al., 2014). Afterward, spermatid elongate to form spermatozoa by spermiogenesis, a morphological transition in which the sperm nucleus transformations involving chromatin reorganization and condensation occur to protect the paternal genetic traits from exogenous and endogenous damage until fertilization (Bao and Bedford, 2016; Govin et al., 2004). Sperm DNA compaction is a stepwise process; substitution of somatic histones by members of the testes-specific histone family, binding of TNPs with a

spermatid nucleus, and finally, replacement of TNPs by PRMs to condense the genetic materials into a highly compact sperm nucleus (Bao and Bedford, 2016; Govin et al., 2004; Rathke et al., 2014). PRMs, the histone-to-PRM transition, and testes-specific histone variations and modifications play important roles in chromatin condensation regulation (Bao and Bedford, 2016; Boskovic and Torres-Padilla, 2013). We discovered that BPA exposure significantly altered the expression levels of H2A, H2B, H3, H4, and TNP1 using Western blotting and IHC staining in testes. Interestingly, we revealed that PRMs are more highly
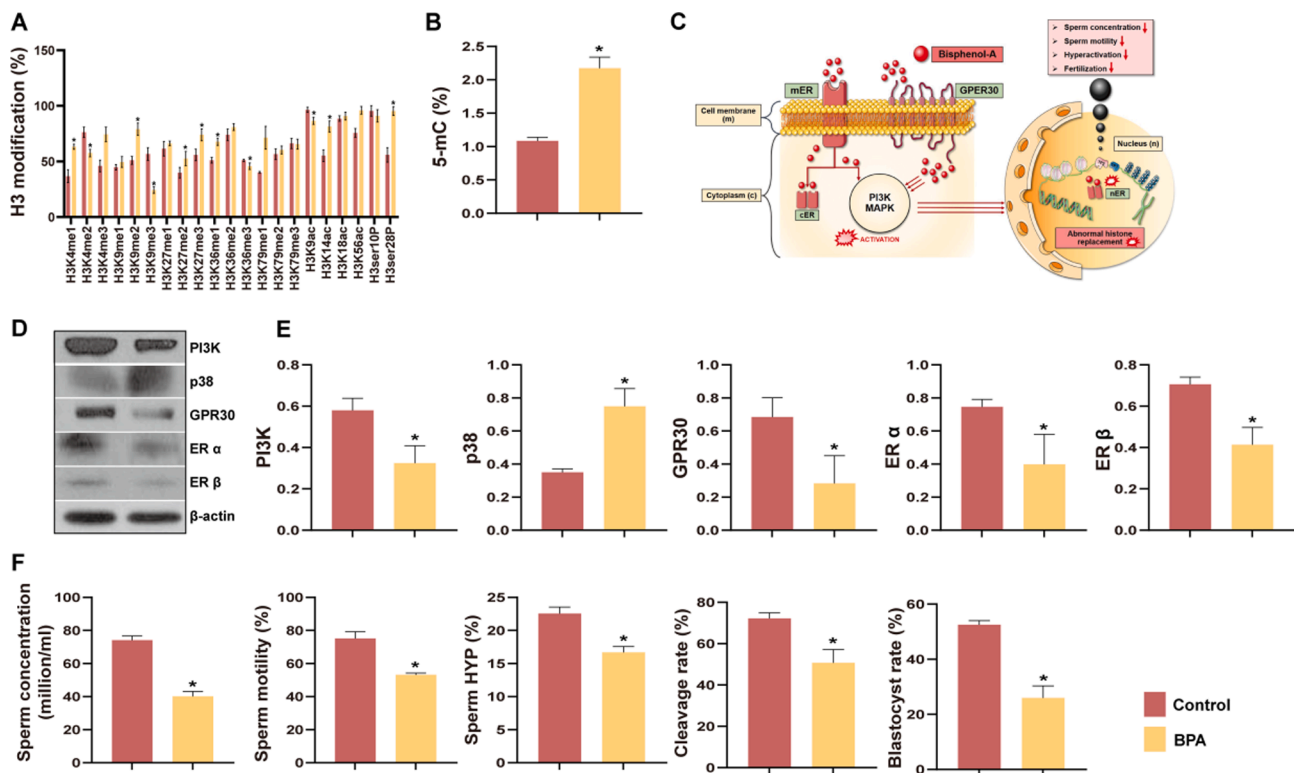
Fig. 5. Effects of BPA on epigenetic modifications, protein expression levels, and male fertility. (**A**) Changes in levels (%) of H3 modifications in testes and (**B**) levels (%) of 5-mC in spermatozoa. (**C**) Schematic of estrogenic signaling regulated by BPA. (**D**) Representative Western Blot images. (**E**) Density of signaling proteins in testis. (**F**) Differences in sperm concentration, motility, hyper-activation, cleavage rate (%), and blastocyst development (%). Data are mean of three replicates ± SD. Asterisks denote significant differences between control and treatment groups at $p < 0.05$ by two-tailed Student's *t*-test. HYP = Hyperactivity.

expressed in the BPA group than in the control group in spermatids but lower in the spermatozoon using IHC staining. It is thus plausible that BPA impacts spermiogenesis from spermatid to spermatozoon, and therefore no proper histone-PRM transition occurred. Although it is difficult to explain why the expression of the histone family was increased along with PRM deficiency, it might have been due to a decrease in various chromatin remodeling proteins required for mediating histone-to-PRM transition during spermiogenesis, according to a recent study (Dumasia et al., 2017a).

Several histone variants/clusters and genes are posited to facilitate the histone-to-PRM transition by incorporating TNPs and contributing to the chromatin structure (Anuar et al., 2019; Barral et al., 2017; Montellier et al., 2013; Padavattan et al., 2015; Shinagawa et al., 2015). Therefore, we evaluated the histone-to-PRM transition-related gene levels to discover the molecular mechanism of BPA exposure to histone-to-PRM transition. Our results showed that two histone variants (HIT2, H2AL2), and one histone cluster (H3F3A) is significantly increased following BPA exposure among 25 genes. H1T2 is a testis-specific gene localized explicitly to the spermatid nuclei but not the nuclei of the spermatozoon. It is crucial for incorporating PRMs and appropriate chromatin condensation (Martianov et al., 2005; Tanaka et al., 2005). H2AL2 is also localized to the elongating and elongated spermatid nuclei but not the spermatozoon. It is also known to contribute to assembling nucleosomes and incorporating TNPs (Barral et al., 2017; Martianov et al., 2005). In addition, H3f3a is a gene that encodes a histone H3.3, localized in all types of germ cells, and has a critical role in chromatin structure and modulating TNP1 removal, and incorporating PRM1 resulting in male infertility (Thakar et al., 2009; Yuen et al., 2014). Therefore, this result has further strengthened our conviction that exposure to BPA elevates the expression levels of histone variants and clusters, which may influence the incorporation of TNPs and PRMs in conjunction with the disruption of histone-to-PRM transition during

spermiogenesis from spermatid to spermatozoon. Because of these dramatic alterations, the modulation of this transition might be considered a potential strategy for discovering biomarkers and therapy for BPA-related fertility damage.

We subsequently identified that BPA exposure significantly decreased the PRM1/PRM2 ratio in both testes and spermatozoa. A certain PRM1/PRM2 ratio is necessary for proper chromatin compaction. An aberrant PRM1/PRM2 ratio can cause sperm DNA damage, embryo death, and infertility (Cho et al., 2001; Hammadeh et al., 2010). Interestingly, the PRM1/PRM2 ratio tends to recover to the normal ratio in spermatozoa (ca. 0.8) compared to the testes (ca. 0.4). It is assumed that the spermatids whose process was not normally performed at the histone-to-PRM transition fell behind in the process of ejaculation due to poor DNA compaction.

PTMs of histones have critical effects on the chromatin conformation by regulating histone–DNA interaction and nucleosome stability. Several types of histone modification have been found to induce the histone-to-PRM transition, including methylation, ubiquitination, acetylation, and phosphorylation (Luense et al., 2016). Histone H3 modification has been frequently associated with the histone-to-PRM transition. For instance, H3K4me1/2/3 and H3K36me3 have been shown to regulate the gene expression levels of TNPs and PRMs, and H3K79me3 has been correlated with histone H4 hyperacetylation to modulate the histone-to-PRM transition (Dottermusch-Heidel et al., 2014; Okada et al., 2007; Wang et al., 2019; Zuo et al., 2018). We found that among 21 different types of H3 modification, 13 of them (H3K4me1/2/3, H3K9me2/3, H3K27me3, H3K36me1/3, H3K79me1, H3Kac, H3K14ac, H3K56ac, and H3S28P) were differentially expressed in testes following BPA exposure. Our experiments corroborate previous studies that BPA exposure influences many histone H3 modifications, such as H3K9me1/2/3 and H3K27me3, during the histone-to-PRM transition (Li et al., 2018; Zhang et al., 2018). These histone

modifications following BPA exposure cause spermatogenesis impairment, abnormal gene translation, and histone-to-PRM transition failure resulting in male infertility.

We established that BPA exposure regulated histone modification, including histone methylation at a specific region, in the testes and significantly increased DNA methylation in spermatozoa. It is thus plausible that BPA induces histone modification on germ cells during spermatogenesis and germ cells differentiate into spermatozoa with an aberrant PRM1/PRM2 ratio and a high percentage of DNA methylation, resulting in abnormal gene modification and male infertility.

Finally, we investigated ERα/β, the primary nuclear receptor targets of BPA to understand better the underlying effect of BPA on the male fertility. ERs regulate the expression of their target genes by a DNA-binding transcription factor that recruits chromatin remodeling factors, affecting nuclear chromatin function. Any changes in ER signaling may lead to adverse consequences for male genital development and reproduction, such as altered hormone levels and thereby alteration of hormone-dependent downstream signaling pathways responsible for gonad development and sometimes impaired fertility. ER signaling may also have a role in epigenetic changes by inducing histone modifications, such as phosphorylation, methylation, and acetylation at ER-specific target gene promoters (Mann et al., 2011). Dumasia et al. reported that treatment with an ERβ agonist reduced DNA methylation at the global level as well as at the H19 differentially methylated region in spermatozoa (Dumasia et al., 2017a). In addition, they revealed that the treatment of ERα agonists increased testicular levels of histone modifications associated with active and repressed chromatin states, besides affecting chromatin remodeling proteins, leading to enhanced histone retention and PRM deficiency in spermatozoa, which could together decrease fertility (Dumasia et al., 2017b). Consistent with previous findings, we found that BPA exposure significantly decreased both ERs. It is possible that BPA exposure regulates gene expression through binding with ERs, which, in turn, activate transcriptional processes, signaling events, or both, leading to an increased histone modification and DNA methylation. In addition, MAPK, PI3K, and GPR30 take part in rapid non-genomic signaling that is extremely effective in response to BPA (Rahman et al., 2022). Although the receptor's activity may vary depending on the stage of development, their dynamic interaction fine-tunes the estrogenic signaling, which in turn supports crucial cellular functions. Herein, we noticed that exposure to BPA regulated the expression of receptors functionally linked to the genomic and non-genomic pathways.

The major finding of this study is that spermatozoa from the testes of mice exposed to BPA exhibited significantly lower fertilization and early embryonic development rates as a consequence of reduced sperm concentration, motility, and HYP. Based on our results, BPA exposure has a detrimental impact on the proteins related to histone-to-PRM transition and may consequently cause abnormal sperm function and male infertility.

5. Conclusions

To our knowledge, this is the first study to investigate the effects of BPA on testes and spermatozoa by determining the changes in the histone-to-PRM transition and male fertility. We demonstrate that BPA exposure alters the core histone proteins, PRMs, TNPs, and histone-to-PRM transition-related genes (histone variants and cluster), which could eventually cause male infertility. The resultant aberrant histone modification in the testes and increased DNA methylation in spermatozoa may significantly impact epigenetic transgenerational inheritance of male infertility. Simultaneously, our results suggest that the PRM1/PRM2 ratio may be a potential and key testicular and spermatozoa biomarker for diagnosing male infertility following BPA exposure. However, testicular cells, such as, Sertoli cells, Leydig cells, and other interstitial cells are present in the testis alongside the germ cells, which makes it more challenging to investigate the process of spermiogenesis.

Therefore, further studies are needed to fully identify the functions and mechanisms of precise BPA dose exposure on specific testicular cell types, such as Leydig, Sertoli, and germ cells, for a better understanding of the action of BPA mechanisms on histone-to-PRM transition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107617.

References

Acerini, C.L., Hughes, I.A., 2006. Endocrine disrupting chemicals: a new and emerging public health problem? Arch. Dis. Child. 91, 633–641.

Anuar, N.D., Kurscheid, S., Field, M., Zhang, L., Rebar, E., Gregory, P., Buchou, T., Bowles, J., Koopman, P., Tremethick, D.J., et al., 2019. Gene editing of the multi-copy H2A.B gene and its importance for fertility. Genome Biol. 20, 23.

Anway, M.D., Rekow, S.S., Skinner, M.K., 2008. Transgenerational epigenetic programming of the embryonic testis transcriptome. Genomics 91, 30–40.

Avendano, C., Franchi, A., Duran, H., Oehninger, S., 2010. DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil. Steril. 94, 549–557.

Bao, J., Bedford, M.T., 2016. Epigenetic regulation of the histone-to-protamine transition during spermiogenesis. Reproduction 151, R55–R70.

Bao, W., Liu, B., Rong, S., Dai, S.Y., Trasande, L., Lehmler, H.J., 2020. Association Between Bisphenol A Exposure and Risk of All-Cause and Cause-Specific Mortality in US Adults. JAMA Netw. Open 3, e2011620.

Barral, S., Morozumi, Y., Tanaka, H., Montellier, E., Govin, J., de Dieuleveult, M., Charbonnier, G., Coute, Y., Puthier, D., Buchou, T., et al., 2017. Histone Variant H2A.L.2 Guides Transition Protein-Dependent Protamine Assembly in Male Germ Cells. Mol. Cell 66, 89–101 e108.

Boskovic, A., Torres-Padilla, M.E., 2013. How mammals pack their sperm: a variant matter. Genes Dev. 27, 1635–1639.

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.

Carlini, T., Paoli, D., Pelloni, M., Faja, F., Dal Lago, A., Lombardo, F., Lenzi, A., Gandini, L., 2017. Sperm DNA fragmentation in Italian couples with recurrent pregnancy loss. Reprod. Biomed. Online 34, 58–65.

Chianese, R., Troisi, J., Richards, S., Scafuro, M., Fasano, S., Guida, M., Pierantoni, R., Meccariello, R., 2018. Bisphenol A in Reproduction: Epigenetic Effects. Curr. Med. Chem. 25, 748–770.

Cho, C., Willis, W.D., Goulding, E.H., Jung-Ha, H., Choi, Y.C., Hecht, N.B., Eddy, E.M., 2001. Haploinsufficiency of protamine-1 or -2 causes infertility in mice. Nat. Genet. 28, 82–86.

Crowe, A.R., Yue, W., 2019. Semi-quantitative Determination of Protein Expression using Immunohistochemistry Staining and Analysis: An Integrated Protocol. Bio Protoc. 9.

Dolinoy, D.C., Jirtle, R.L., 2008. Environmental epigenomics in human health and disease. Environ. Mol. Mutagen. 49, 4–8.

Dottermusch-Heidel, C., Klaus, E.S., Gonzalez, N.H., Bhushan, S., Meinhardt, A., Bergmann, M., Renkawitz-Pohl, R., Rathke, C., Steger, K., 2014. H3K79 methylation directly precedes the histone-to-protamine transition in mammalian spermatids and is sensitive to bacterial infections. Andrology 2, 655–665.

Dumasia, K., Kumar, A., Deshpande, S., Balasinor, N.H., 2017a. Estrogen signaling, through estrogen receptor beta, regulates DNA methylation and its machinery in male germ line in adult rats. Epigenetics 12, 476–483.

Dumasia, K., Kumar, A., Deshpande, S., Balasinor, N.H., 2017b. Estrogen, through estrogen receptor 1, regulates histone modifications and chromatin remodeling during spermatogenesis in adult rats. Epigenetics 12, 953–963.

Francis, S., Yelumalai, S., Jones, C., Coward, K., 2014. Aberrant protamine content in sperm and consequential implications for infertility treatment. Hum. Fertil. (Camb.) 17, 80–89.

Govin, J., Caron, C., Lestrat, C., Rousseaux, S., Khochbin, S., 2004. The role of histones in chromatin remodelling during mammalian spermiogenesis. Eur. J. Biochem. 271, 3459–3469.

Hammadeh, M.E., Hamad, M.F., Montenarh, M., Fischer-Hammadeh, C., 2010. Protamine contents and P1/P2 ratio in human spermatozoa from smokers and non-smokers. Hum. Reprod. 25, 2708–2720.

Hengstler, J.G., Foth, H., Gebel, T., Kramer, P.J., Lilienblum, W., Schweinfurth, H., Volkel, W., Wollin, K.M., Gundert-Remy, U., 2011. Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A. Crit. Rev. Toxicol. 41, 263–291.

Hess, R.A., Renato de Franca, L., 2008. Spermatogenesis and cycle of the seminiferous epithelium. Adv. Exp. Med. Biol. 636, 1–15.

Hogarth, C.A., Griswold, M.D., 2013. Immunohistochemical approaches for the study of spermatogenesis. Methods Mol. Biol. 927, 309–320.

Hope, E., Reed, D.R., Moilanen, L.H., 2016. Potential confounders of bisphenol-a analysis in dental materials. Dent. Mater. 32, 961–967.

Ikezuki, Y., Tsutsumi, O., Takai, Y., Kamei, Y., Taketani, Y., 2002. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum. Reprod. 17, 2839–2841.

Kubwabo, C., Kosarac, I., Stewart, B., Gauthier, B.R., Lalonde, K., Lalonde, P.J., 2009. Migration of bisphenol A from plastic baby bottles, baby bottle liners and reusable polycarbonate drinking bottles. Food Addit. Contam. Part A Chem. Anal. Control Expo Risk Assess. 26, 928–937.

Li, Y., Duan, F., Zhou, X., Pan, H., Li, R., 2018. Differential responses of GC1 spermatogonia cells to high and low doses of bisphenol A. Mol. Med. Rep. 18, 3034–3040.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408.

Luense, L.J., Wang, X., Schon, S.B., Weller, A.H., Lin Shiao, E., Bryant, J.M., Bartolomei, M.S., Coutifaris, C., Garcia, B.A., Berger, S.L., 2016. Comprehensive analysis of histone post-translational modifications in mouse and human male germ cells. Epigenetics Chromatin 9, 24.

Mann, M., Cortez, V., Vadlamudi, R.K., 2011. Epigenetics of estrogen receptor signaling: role in hormonal cancer progression and therapy. Cancers (Basel) 3, 1691–1707.

Martianov, I., Brancorsini, S., Catena, R., Gansmuller, A., Kotaja, N., Parvinen, M., Sassone-Corsi, P., Davidson, I., 2005. Polar nuclear localization of H1T2, a histone H1 variant, required for spermatid elongation and DNA condensation during spermiogenesis. Proc. Natl. Acad. Sci. USA 102, 2808–2813.

Montellier, E., Boussouar, F., Rousseaux, S., Zhang, K., Buchou, T., Fenaille, F., Shiota, H., Debernardi, A., Hery, P., Curtet, S., et al., 2013. Chromatin-to-nucleoprotamine transition is controlled by the histone H2B variant TH2B. Genes Dev. 27, 1680–1692.

Ni, K., Spiess, A.N., Schuppe, H.C., Steger, K., 2016. The impact of sperm protamine deficiency and sperm DNA damage on human male fertility: a systematic review and meta-analysis. Andrology 4, 789–799.

Nili, H.A., Mozdarani, H., Aleyasin, A., 2009. Correlation of sperm DNA damage with protamine deficiency in Iranian subfertile men. Reprod. Biomed. Online 18, 479–485.

Okada, Y., Scott, G., Ray, M.K., Mishina, Y., Zhang, Y., 2007. Histone demethylase JHDM2A is critical for Tnp1 and Prm1 transcription and spermatogenesis. Nature 450, 119–123.

Oliva, R., Ballesca, J.L., 2012. Altered histone retention and epigenetic modifications in the sperm of infertile men. Asian J. Androl. 14, 239–240.

Oliva, R., Dixon, G.H., 1991. Vertebrate protamine genes and the histone-to-protamine replacement reaction. Prog. Nucleic Acid Res. Mol. Biol. 40, 25–94.

Pacheco, S.E., Houseman, E.A., Christensen, B.C., Marsit, C.J., Kelsey, K.T., Sigman, M., Boekelheide, K., 2011. Integrative DNA methylation and gene expression analyses identify DNA packaging and epigenetic regulatory genes associated with low motility sperm. PLoS ONE 6, e20280.

Padavattan, S., Shinagawa, T., Hasegawa, K., Kumasaka, T., Ishii, S., Kumarevel, T., 2015. Structural and functional analyses of nucleosome complexes with mouse histone variants TH2a and TH2b, involved in reprogramming. Biochem. Biophys. Res. Commun. 464, 929–935.

Pang, W.K., Kang, S., Ryu, D.Y., Rahman, M.S., Park, Y.J., Pang, M.G., 2020. Optimization of sperm RNA processing for developmental research. Sci. Rep. 10, 11606.

Park, Y.J., Rahman, M.S., Pang, W.K., Ryu, D.Y., Kim, B., Pang, M.G., 2020. Bisphenol A affects the maturation and fertilization competence of spermatozoa. Ecotoxicol. Environ. Saf. 196, 110512.

Park, Y.J., Rahman, M.S., Pang, W.K., Ryu, D.Y., Adegoke, E.O., Rahman, M.S., Pang, M.G., 2021. Bisphenol A exposure increases epididymal susceptibility to infection in mice. Ecotoxicol. Environ. Saf. 208, 111476.

Platts, A.E., Dix, D.J., Chemes, H.E., Thompson, K.E., Goodrich, R., Rockett, J.C., Rawe, V.Y., Quintana, S., Diamond, M.P., Strader, L.F., et al., 2007. Success and failure in human spermatogenesis as revealed by teratozoospermic RNAs. Hum. Mol. Genet. 16, 763–773.

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.

Rahman, M.S., Pang, W.K., Ryu, D.Y., Park, Y.J., Pang, M.G., 2020. Multigenerational and transgenerational impact of paternal bisphenol A exposure on male fertility in a mouse model. Hum. Reprod. 35, 1740–1752.

Rahman, M.S., Adegoke, E.O., Pang, M.G., 2021a. Drivers of owning more BPA. J. Hazard. Mater. 417, 126076.

Rahman, M.S., Pang, W.K., Ryu, D.Y., Park, Y.J., Ryu, B.Y., Pang, M.G., 2021b. Multigenerational impacts of gestational bisphenol A exposure on the sperm function and fertility of male mice. J. Hazard. Mater. 416, 125791.

Rahman, M.S., Pang, W.K., Amjad, S., Ryu, D.Y., Adegoke, E.O., Park, Y.J., Pang, M.G., 2022. Hepatic consequences of a mixture of endocrine-disrupting chemicals in male mice. J. Hazard. Mater. 436, 129236.

Rathke, C., Baarends, W.M., Awe, S., Renkawitz-Pohl, R., 2014. Chromatin dynamics during spermiogenesis. BBA 1839, 155–168.

Rezg, R., El-Fazaa, S., Gharbi, N., Mornagui, B., 2014. Bisphenol A and human chronic diseases: current evidences, possible mechanisms, and future perspectives. Environ. Int. 64, 83–90.

Rochester, J.R., 2013. Bisphenol A and human health: a review of the literature. Reprod. Toxicol. 42, 132–155.

Roosen-Runge, E.C., 1962. The process of spermatogenesis in mammals. Biol. Rev. Camb. Philos. Soc. 37, 343–377.

Ryu, D.Y., Kim, K.U., Kwon, W.S., Rahman, M.S., Khatun, A., Pang, M.G., 2017a. Peroxiredoxin activity is a major landmark of male fertility. Sci. Rep. 7, 17174.

Ryu, D.Y., Rahman, M.S., Pang, M.G., 2017b. Determination of Highly Sensitive Biological Cell Model Systems to Screen BPA-Related Health Hazards Using Pathway Studio. Int. J. Mol. Sci. 18.

Schneider, S., Balbach, M., Jan, F.J., Fietz, D., Nettersheim, D., Jostes, S., Schmidt, R., Kressin, M., Bergmann, M., Wachten, D., et al., 2016. Re-visiting the Protamine-2 locus: deletion, but not haploinsufficiency, renders male mice infertile. Sci. Rep. 6, 36764.

Schug, T.T., Janesick, A., Blumberg, B., Heindel, J.J., 2011. Endocrine disrupting chemicals and disease susceptibility. J. Steroid Biochem. Mol. Biol. 127, 204–215.

Shinagawa, T., Huynh, L.M., Takagi, T., Tsukamoto, D., Tomaru, C., Kwak, H.G., Dohmae, N., Noguchi, J., Ishii, S., 2015. Disruption of Th2a and Th2b genes causes defects in spermatogenesis. Development 142, 1287–1292.

Song, X., Miao, M., Zhou, X., Li, D., Tian, Y., Liang, H., Li, R., Yuan, W., 2019. Bisphenol A Exposure and Sperm ACHE Hydroxymethylation in Men. Int. J. Environ. Res. Public Health 16.

Steger, K., Klonisch, T., Gavenis, K., Drabent, B., Doenecke, D., Bergmann, M., 1998. Expression of mRNA and protein of nucleoproteins during human spermiogenesis. Mol. Hum. Reprod. 4, 939–945.

Stiavnicka, M., Garcia-Alvarez, O., Ulcova-Gallova, Z., Sutovsky, P., Abril-Parreno, L., Dolejsova, M., Rimnacova, H., Moravec, J., Hosek, P., Losan, P., et al., 2020. H3K4me2 accompanies chromatin immaturity in human spermatozoa: an epigenetic marker for sperm quality assessment. Syst. Biol. Reprod. Med. 66, 3–11.

Tanaka, H., Iguchi, N., Isotani, A., Kitamura, K., Toyama, Y., Matsuoka, Y., Onishi, M., Masai, K., Maekawa, M., Toshimori, K., et al., 2005. HANP1/H1T2, a novel histone H1-like protein involved in nuclear formation and sperm fertility. Mol. Cell. Biol. 25, 7107–7119.

Thakar, A., Gupta, P., Ishibashi, T., Finn, R., Silva-Moreno, B., Uchiyama, S., Fukui, K., Tomschik, M., Ausio, J., Zlatanova, J., 2009. H2A.Z and H3.3 histone variants affect nucleosome structure: biochemical and biophysical studies. Biochemistry 48, 10852–10857.

Tyl, R.W., 2009. Basic exploratory research versus guideline-compliant studies used for hazard evaluation and risk assessment: bisphenol as a case study. Environ. Health Perspect. 117, 1644–1651.

Wang, X., Kang, J.Y., Wei, L., Yang, X., Sun, H., Yang, S., Lu, L., Yan, M., Bai, M., Chen, Y., et al., 2019. PHF7 is a novel histone H2A E3 ligase prior to histone-to-protamine exchange during spermiogenesis. Development 146.

Wdowiak, A., Bakalczuk, S., Bakalczuk, G., 2015. The effect of sperm DNA fragmentation on the dynamics of the embryonic development in intracytoplasmatic sperm injection. Reprod. Biol. 15, 94–100.

Welshons, W.V., Nagel, S.C., vom Saal, F.S., 2006. Large effects from small exposures. III. Endocrine mechanisms mediating effects of bisphenol A at levels of human exposure. Endocrinology 147, S56–S69.

Yuen, B.T., Bush, K.M., Barrilleaux, B.L., Cotterman, R., Knoepfler, P.S., 2014. Histone H3.3 regulates dynamic chromatin states during spermatogenesis. Development 141, 3483–3494.

Zhang, T., Zhou, Y., Li, L., Zhao, Y., De Felici, M., Reiter, R.J., Shen, W., 2018. Melatonin protects prepuberal testis from deleterious effects of bisphenol A or diethylhexyl phthalate by preserving H3K9 methylation. J. Pineal Res. 65, e12497.

Zheng, H., Zhou, X., Li, D.K., 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 6, e0178535.

Zuo, X., Rong, B., Li, L., Lv, R., Lan, F., Tong, M.H., 2018. The histone methyltransferase SETD2 is required for expression of acrosin-binding protein 1 and protamines and essential for spermiogenesis in mice. J. Biol. Chem. 293, 9188–9197.